1999

Reduction of Endogenous Bacteria Associated With Catfish (Ictalurus Punctatus) Fillets Using the Grovac Process.

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REDUCTION OF ENDOGENOUS BACTERIA ASSOCIATED WITH CATFISH (ICTALURUS PUNCTATUS) FILLETS USING THE GROVAC PROCESS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Food Science

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December 1999

DEDICATION

This work is dedicated to my wife, Lupita, my daughter, María, my son, Juan José, my son in-law, Marlon, and my granddaughter, Alejandra for their love and support during my entire study here in the United States.

This work is also dedicated to my grandparents, Rosa and José Antonio, my parents, María and Vicente, my sisters, Graciela, Gladys, Rosa, Gloria and Lupe, and my brothers, Carlos, José and Patricio for their support and encouragement.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to his advisor, Dr. Wanda J. Lyon for her indispensable guidance, encouragement and personal advise. Appreciation is extended to the members of the Advisory Committee, Dr. Evelina Cross, Dr. Maren Hegsted, Dr. Witoon Prinyawiwatkul, Dr. Gary Cadd, and Dr. Ramu Rao. Special thanks to the Commissioner of the Louisiana Department of Agriculture and Forestry, Tony's Live Seafoods, and Bill Groves for their assistance in providing monetary assistance, catfish samples and equipment, respectively.

Gratitude is expressed to La Fundación para la Ciencia y Tecnología de Ecuador and La Universidad Técnica de Ambato for their financial assistance during his doctoral program. The author thanks to Christina Korinek and Staff of LASPAU for their support and friendship.

TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

Fresh catfish (*Ictalurus punctatus*) fillets (CF) can serve as vehicles for spoilage and pathogenic bacteria. Concern for the microbiological safety of chilled CF has grown lately because psychrotrophic pathogens can survive and grow at refrigeration temperatures. The Grovac method, a new, patented (U.S. 5,543,163) process is being used in an attempt to reduce the growth of pathogens and spoilage microorganisms in CF. This process involves the using of a process solution of ascorbic acid (AA) and sodium chloride (NaCl), vacuum and tumbling. Specific objectives of this study included the isolation and identification of endogenous microflora associated with fresh whole catfish (WC) and CF; and the evaluation of the Grovac process for reducing microbial populations on CF.

A total of fifty-one bacterial isolates were isolated and identified from WC and CF using selective and nonselective media, phenotypic tests, and the Vitek^{TM} System (bioMérieux). Psychrotrophic foodborne pathogens included: *Aeromonas hydrophila, Escherichia coli, Listeria* sp., *Plesiomonas shigelloides, Proteus* sp., *Staphylococcus aureus,* and *Vibrio parahaemolyticus.* High APC (2.6×10^7 CFU/g) and *E. coli* (3.2×10^3 CFU/g) counts for CF indicated that fillets were heavily contaminated during processing of catfish.

The Grovac process showed that treatment combinations of AA (0.4, 0.8 and 1.2%) and NaCl (0.2, 0.4 and 0.6%) under the following experimental conditions (400 g of catfish fillets, 2,000 ml process solution, 28 in. Hg vacuum, 8 rpm tumbler rotation, and 8 min tumbling time) resulted in a 1.2 to 2.3 CFU/g log reduction of microbial counts in CF. The effectiveness of the process may be related to the synergistic effect of tumbling, AA, NaCl and vacuum. In addition, *A. hydrophila* grew in CF treated with the Grovac process and aerobically stored at 4°C for 7 days.

The results suggested that the Grovac process could be used as an alternative processing procedure to reduce microbial populations on CF and be useful to improve the shelf-life and food safety of the product. Microbiological data from this study will be used for the development of a hazard analysis for the implementation of the Hazard Analysis Critical Control Point (HACCP) program for processed CF.

INTRODUCTION

Aquacultured channel catfish (*Ictalurus punctatus*) fillets are marketed as a fresh (ice-packed) product. Sales of fresh catfish in 1998, at 167 million pounds, represented 60 percent of total sales and were up 6 percent from 1997 (USDA; United States Department of Agriculture, 1999). Unfortunately, fresh catfish fillets can serve as vehicles for spoilage and pathogenic bacteria which cause spoilage of the product and human diseases (Fernandez *et al.*, 1997). During processing of catfish (e.g., deheading, skinning, eviscerating), the microorganisms in the skin, the gills and the gut can be spread onto the processing equipment, the workers, and the flesh of the fillet. Hence, the normal sterile flesh can be inoculated with millions of bacteria. The microbial flora on catfish fillets leaving the processing plant may be different from that of whole catfish entering the plant (Banwart, 1989; Bonnell, 1994; Garthwaite, 1997; Inglis *et al.*, 1993).

Traditionally, the growth of spoilage bacteria on catfish fillets has been prevented at refrigeration temperatures (Huang *et al.*, 1994; Reddy *et al.*, 1997; Reed *et al.*, 1983). These preservation methods normally result in a short shelf-life of 5-10 days. After this period of time, catfish fillets deteriorate primarily through microbiological spoilage. However, concern for the microbiological safety of chilled catfish fillets has grown in the last few years. Fernandez *et al.* (1998b) and Leung *et al.* (1992a) reported that psychrotrophic pathogens (e.g., *Listeria monocytogenes*, *Aeromonas hydrophila*) can survive and grow at refrigeration temperatures. Thus the use of refrigeration can no longer be deemed sufficient to keep catfish fillets safe from bacterial hazards. In addition, the sale of catfish fillets containing psychrotrophic pathogens as their natural flora in supermarket display and other outlets could cause the possible cross-contamination of the fresh fillets with cooked ready-to-eat products during market handling or in the home refrigerator (Fernandez *et al.*, 1998b). Therefore, food scientists are developing several control methods (e.g., modified atmosphere packaging (MAP), antimicrobial preservatives) to improve the shelf-life and safety of refrigerated

catfish products (Fernandez *et al.*, 1998a; Kim *et al.*, 1995a; Kim and Hearnsberger, 1994; Kim *et al.*, 1995b; Marshall and Kim, 1996; Przybylski *et al.*, 1989; Silva *et al.*, 1993; Silva and White, 1994; Williams *et al.*, 1995). Currently, the Grovac method, a new, patented process is being used in an attempt to enhance the shelf-life of catfish fillets.

Until today, the efficiency of the Grovac process to reduce the growth of pathogens and spoilage microorganisms in fresh catfish fillets has not been thoroughly investigated to assure the shelf-life and safety of the product. Base-line studies on the absence and presence of foodborne pathogens are required to evaluate processing techniques on reduction of endogenous pathogens associated with fresh catfish fillets at refrigerated temperatures. In addition to processing techniques for eliminating pathogens associated with catfish, microbiological data are valuable for the hazard analysis needed during HACCP implementation of processed catfish fillets. Current regulations require seafood processors to develop and implement HACCP programs to ensure product safety (Lyon, 1999). There is also evidence that some psychrotrophic pathogens associated with outbreaks can grow at refrigeration temperatures and may be part of the natural microflora of fresh catfish fillets. Pathogens may multiply to an undesirable level on refrigerated fresh catfish during a normal storage period. It is also possible that the Grovac process may influence the growth rate of endogenous microflora of catfish fillets. Consequently, the specific objectives of this study were as follows: (1) To evaluate base-line endogenous microflora associated with fresh whole catfish (*Ictalurus punctatus*) and fresh catfish fillets; (2) To evaluate the reduction of endogenous microflora of fresh catfish fillets using the Grovac processing method; and (3) To evaluate microbiological shelf-life of refrigerated fresh catfish fillets at $4^{o}C$ treated with the Grovac process.

REVIEW OF LITERATURE

Commercial Catfish Culture

Channel catfish. The channel catfish (*Ictalurus punctatus*) is the most important species of aquatic animal commercially cultured in the United States. It belongs to the family Ictaluridae, order Siluriformes. Members of the order Siluriformes are found in fresh and salt water worldwide. Besides the channel catfish, other species that have been cultured or have some potential for commercial production, including blue catfish, white catfish, the bullhead, yellow bullhead, black bullhead, and flathead catfish (Wellborn, 1992; Wellborn and Tucker, 1985).

Channel catfish possess a combination of desirable qualities for commercial production such as good market value, ability to reproduce in captivity, rapid growth, acceptance of prepared feeds, resistance to diseases, and tolerance to variable conditions of dissolved oxygen and temperature of the water. The benefits of aquaculture, or fish farming, are as follows: (1) supply and price of catfish can be controlled effectively; and (2) high quality can be maintained because the production and harvest conditions are controlled and farmed catfish go into the processing plant alive (Lovell, 1991).

Physical characteristics. Like all native North American catfishes, a channel catfish has a body that is cylindrical in cross-section and the skin is without scales. Fins are soft-rayed except for the dorsal and pectoral fins which have sharp, hard spines. One conspicuous characteristic of all catfish is the presence of barbels around the mouth. The barbels are arranged in a definite pattern: four under the jaw and one on each tip of the maxilla (upper jaw). The channel catfish is the only spotted catfish with a deeply forked tail (Wellborn, 1992; Wellborn and Tucker, 1985). The flesh of the channel catfish is white, is free of intramuscular bones, and has a mild flavor (Lovell, 1991).

Nutritional value. Proximate analysis of channel catfish are: moisture, 76%; protein, 17.8%; fat, 6%; and ash 1.2% (Ammerman, 1985). The protein percentage in catfish flesh ranges from 14 to 18%, varying inversely with fat content. Fat content in catfish fillets varies from 6 to 12% (Lovell, 1991).

Geographical distribution. Most channel catfish farming is conducted in large earthen ponds in the southeastern United States, a region with a warm-temperate climate that is characterized by wide seasonal climatic and environmental variation. Catfish grow best in warm water with optimum growth occurring at temperatures between 26 to 30^{o}C (Stickney, 1991; Wellborn and Tucker, 1985). Channel catfish are freshwater fish but they can thrive in brackish water (Wellborn, 1992). The area with the greatest concentration of production has centered around the region along the Mississippi River, especially in the delta section of the states of Mississippi and Arkansas (Lee, 1991).

Catfish production. The major catfish producing states are Alabama, Arkansas, Louisiana, and Mississippi. Mississippi produces most of the farm-raised catfish in the United States (USDA, 1999). Commercial production of catfish in the U.S. has increased at a phenomenal rate in the past several years. Increased production rates of catfish have been influenced by proper husbandry, control over water quality, availability of nutritionally balanced feeds, improved harvesting methodology, and implementation of the selective harvest strategy (Stickney, 1991). Farm-raised catfish processed during 1998 totaled 564 million pounds, a new record, 8 percent above the 525 million pounds processed in 1997, the previous record. Catfish growers had sales of 469 million dollars in 1998 and the average price paid to producer was 74.3 cents per pound (USDA, 1999).

Catfish processing. Like production, the catfish processing industry has also increased dramatically to keep up with rapid changes in supply and demand. Net pounds of processed catfish sold in 1998 totaled 281 million pounds (USDA, 1999). Processing involves preparing catfish for purchase by retail markets or by consumers.

The main steps in processing include the following: checking for off-flavor, grading, stunning, deheading, skinning, eviscerating, washing, cutting, packaging, storing, and transportation (Lee, 1991; Ammerman, 1985). Channel catfish are processed in plants near the farms primarily into fillets and whole dressed fish without the head, skin, and viscera. Sales of fillets in 1998, at 167 million pounds, represented 60 percent of total sales. Other catfish products (e.g., whole dressed, steaks, nuggets) account for the remaining 40 percent of total sales (USDA, 1999). All these forms are marketed as fresh and frozen products.

Catfish products are sold by processors to institutional and retail markets. The majority of catfish products are packaged and delivered fresh (ice packaged), individually quick frozen or chill packed. Fresh catfish intended for immediate consumption are packaged in a variety of ways to meet specifications of the customers. For example, fillets may be packaged in one or more polybags on ice in a wax-coated corrugated box. When customers request ice pack, the fillets are placed in wax-coated corrugated boxes with holes at the bottom of the side panels to allow for drainage as the ice melts. For a chill packed product, fillets are packaged in styrofoam trays, with soaker pads on the bottom, and covered with a polyfilm. Trays are then placed in corrugated boxes without ice and move into a blast freezer at temperatures between -4 and -1 ^{o}C for a short period of time to form a crust-freezing effect. This product is shipped and sold through retail outlets as fresh fish. Frozen fillets may be individually quick frozen by equipment such as spiral or tunnel freezers (McGilberry *et al.*, 1989).

Off-flavor. One important attribute for successful marketing of farm-raised channel catfish is its mild flavor and the lack of fishy odor that is typical for marine and wild freshwater fish. Unfortunately, catfish may acquire flavors perceived as unacceptable by the consumer when they are raised in ponds. Such objectionable flavors are known as "off-flavors" (Van Der Ploeg, 1992). In fact, off-flavor is one of the major problems in catfish culture and it is estimated to cost catfish farmers in excess

of 50 million dollars annually (Perschbacher, 1995). Catfish found to have off-flavor are either not harvested or are maintained on feed in good quality water quality management until they are again considered to be "on-flavor" (Johnsen *et al.*, 1996).

Odorous compounds in pond water are responsible for off-flavors that develop in the fish prior harvesting. Sources of odorous compounds in catfish ponds include algae, microorganisms, fish waste products, and pollutants (e.g., diesel fuel, pesticides) (Van Der Ploeg, 1992). It has been stated that certain microbes (Johnsen *et al.*, 1996) and larger cyanobacteria (blue-green algae) (Perschbacher, 1995) produce geosmin (1α, 10β–dimethyl-9α–decalol) and 2-methylisoborneol (MIB). Low concentrations of either geosmin or MIB impart intense muddy/earthy off-flavors in both the water and commercially produced catfish. These compounds are absorbed by fish through the gills and accumulate in the flesh (Van Der Ploeg, 1992). Several approaches have been proposed to reduce the risk associated with the high incidence of off-flavor such as an effective algal management (Perschbacher, 1995).

Bacterial diseases of catfish. Besides off-flavor, the other major problem in channel catfish farming is disease (Perschbacher, 1995). The interactions between fish and the microorganisms that may be harmless or beneficial under natural conditions often result in disease problems in hatchery fish because of the added stress from the physical, chemical, or biological challenges inherent to intensive culture systems. Such challenges elicit a catecholamine and corticosteroid cascade that brings about a series of cardiovascular, respiratory, and other secondary physiological changes intended to help the fish avoid or escape from the challenge in question (Wedemeyer, 1997). Stress, either physiological or environmental, is a potent suppressor of the immune system, which in turn can predispose the fish to the disease (Reddington and Lightner, 1994). The harmful effects also include varying degrees of growth suppression and reproductive dysfunction (Wedemeyer, 1997).

Stress factors such as unfavorable water quality conditions and fish culture practices cause some bacterial diseases. Adverse water quality conditions, including low dissolved oxygen concentration, high ammonia and carbon dioxide concentrations, unfavorable pH, pollution, and temperature variation. Fish culture practices identified as contributory include overcrowding, excessive pond fertilization, overfeeding, algal blooms, handling, and transportation (Reddington and Lightner, 1994; Stickney, 1991; Wedemeyer, 1997). The main stress-mediated bacterial diseases in catfish culture include motile *Aeromonas* septicemias (*Aeromonas hydrophila* and others), enteric septicemia (*Edwardsiella ictaluri*), emphysematous putrefactive (*Edwardsiella tarda*), and columnaris (*Flexibacter columnaris*) (Wedemeyer, 1997; Stickney, 1991; MacMillan, 1985). Avoidance of stress are the best means that intensive catfish culturists have of reducing the incidence of diseases (Stickney, 1991).

Bacteria in Fresh Catfish

Natural microflora. Hundreds of species of bacteria are found in catfish ponds. Most of them are free-living and perform beneficial functions such as the decomposition of organic matter. The number and types of microorganisms found on freshly caught catfish are influenced by the geographical location of the catch, the season, method of harvest, and environment (Nickelson and Finne, 1992). For example, Leung *et al.* (1992) reported that the bacterial concentration associated with channel catfish reflected the bacterial levels in the pond environment. They found that there were no differences in the fecal streptococci (from 0.78 to 3.49 log CFU/g) and the fecal coliforms (from 2.10 to 4.81 log CFU/g) of the water, sediment, and fish viscera.

Largest concentrations of microorganisms are found in the intestine, gills, and surface slime of catfish. The microfloras in the alimentary tracts of freshwater fish contain primarily species of *Aeromonas* and *Plesiomonas*, and representatives of the family *Enterobacteriaceae*. These bacteria are distributed widely in freshwater environments, and are believed to survive and multiply under the selective conditions of

the alimentary tract in fish. Other bacterial species, which are considered to be derived from water and diets, include *Acinetobacter, Bacillus, Flavobacterium, Micrococcus, Moraxella* and *Pseudomonas*. Whereas the microflora in the intestines are reported to consist predominantly of fermentative bacteria, including *Aeromonas* and Enterobacteriaceae representatives. The dominant bacteria associated with the skin and gills are considered to be *Acinetobacter, Flavobacterium, Moraxella* and *Pseudomonas*. In addition, *Vibrio* sp. are common aquatic bacteria and typically are isolated as endogenous catfish flora (Austin and Austin, 1989; Davies, 1997).

Spoilage bacteria. Fish spoilage is a complex process involving microbiological and nonmicrobiological processes. Nonmicrobiological deterioration is caused by endogenous proteolytic enzymes which are concentrated in the head and viscera and attack these organs and surrounding tissues after death. This deterioration may be compounded by deterioration due to oxygenation of unsaturated fatty acids causing loss of flavor and development of rancidity. Enzymatic spoilage is followed by the growth of microorganisms which invade the flesh of the fish, causing breakdown of the tissues and a general deterioration of the product. During processing of catfish (e.g., deheading, skinning, eviscerating), the microorganisms in the surface slime layer, the gills and the gut can be spread onto the processing equipment, the workers, and the flesh of the fillet. Hence, the normal sterile flesh can be inoculated with millions of bacteria. The microbial flora on the fish leaving the processing plant may be different from that of fish entering the plant (Banwart, 1989; Bonnell, 1994; Garthwaite, 1997; Inglis *et al.*, 1993).

The number and types of microorganisms in catfish fillets are determined by the natural microflora of catfish and the manner in which the fish was handled during harvesting, processing and storage (Nickelson and Finne, 1992). The primary specific spoilage organisms of fresh fish stored aerobically at refrigeration temperatures are *Aeromonas* sp., *Pseudomonas* sp., and *S. putrefaciens* (Davies, 1997). These bacteria

are capable of causing spoilage because of two important characteristics. First, they are psychrotrophic, and thus multiply at refrigeration temperatures. Secondly, they attack various substances in the fish tissue to produce compounds associated with off-flavors and off-odors. These compounds are reported to be methyl mercaptan, dimethyl disulfide, dimethyl trisulfide, 3-methyl-1-butanal, trimethylamine, and ethyl esters of acetate, butyrate and hexanoate (Nickelson and Finne, 1992). Trimethylamine is also produced by species of *Aeromonas*, *Citrobacter*, *Hafnia alvei*, *Proteus* (*Morganella*) *morganii*, *Proteus vulgaricus* and *Vibrio* (Austin and Austin, 1989). Spoilage microorganisms do not represent a health hazard, but they are associated with spoilage and the economic loss of the food product.

Bacterial pathogens. Only few species of bacteria are pathogens and cause disease only when the immune system of the catfish is suppressed. For example, one of the most common bacterial diseases of channel catfish, enteric septicemia, is caused by an obligate pathogen *Edwardsiella ictaluri*. Obligate pathogens require a living host in order to grow and reproduce. Other stress-mediated fish diseases caused by facultative bacterial pathogens such as *Aeromonas hydrophila*, *A. sobria*, *Pseudomonas fluorescens*, *Flexibacter columnaris* and *Edwardsiella tarda* are the most frequently encountered in aquaculture. Facultative pathogens do not require aquatic animal life forms as hosts and they can obtain sufficient nutrients to live and reproduce while attached to aquatic plants, suspended organic material, or even sediment particles. They are also normally ubiquitous in natural waters (MacMillan, 1985; Stickney, 1991; Tucker and Robinson, 1990; Wedemeyer, 1997).

Human infections may be caused by bacteria endogenous to fish. According to Austin and Austin (1989), bacterial pathogens, which may be transferred from fish or their holding water to human beings, include: *A. hydrophila* (septicemia, diarrhea), *Campylobacter jejuni* (gastroenteritis), *Clostridium botulinum* (type E) (botulism), *Edwardsiella tarda* (diarrhea), *Erysipelothrix rhusiopathiae* (fish rose), *Leptospira*

interrogans (leptospirosis), *Mycobacterium fortuitom/marinum* (mycobacteriosis), *Plesiomonas shigelloides* (gastroenteritis), *Pseudomonas aeruginosa* (wound infections), *P. fluorescens* (wound infections), *Salmonella* sp. (food poisoning), and *Vibrio parahaemolyticus* (food poisoning).

Some previous studies have shown that there is a relationship between pathogenic bacteria and catfish contamination. For example, a survey of retail samples of raw catfish fillets conducted in nine southeastern states indicates that the overall incidence of *Salmonella* is 5% (Andrews *et al.*, 1977). *L. monocytogenes* and *Salmonella* sp. are found in channel catfish under aerobic and 25% CO_2 environments at 8°C (Silva and White, 1994). *A. hydrophila* strains associated with human gastroenteritis are capable of growing in foods (Palumbo *et al.*, 1985) and packaged channel catfish fillets (Leung *et al.*, 1992a) at refrigeration temperatures considered adequate for preventing the growth of foodborne pathogens. The main pathogens associated with contaminated fish are *Clostridium botulinum, Escherichia coli, Listeria monocytogenes, Salmonella* sp., and *Vibrio* sp. (Farber, 1991; Jay, 1992). However, there have been no reported illnesses of foodborne disease linked to the consumption of catfish. Seafoods have been implicated as one of the leading foods in the transmission of foodborne outbreaks for the period 1977-1984 in the U.S. with an incidence rate of 24.8% (Bryan, 1988). Similarly, seafoods again contributed approximately 22.4% of the foodborne outbreaks in 1983-1987 (Dillon and Patel, 1992).

Control Methods

Spoilage and pathogenic bacteria microorganisms are undesirable due to their ability to cause food spoilage and foodborne diseases. Several control methods, individually or in combinations, are used to prevent and minimize microbial contamination of catfish products and to inhibit the growth of or destroy microbial contamination.

Low temperatures. Traditionally, catfish products are stored at low temperatures to extend their shelf-life. It has been stated that the metabolic activities, enzymatic reactions and growth rates of microorganisms slow down as the temperature is reduced (Ray, 1996). Catfish stored at refrigeration temperatures normally result in a short shelf-life of 5-10 days (Reddy *et al.*, 1997); however, a longer shelf-life is achieved by freezing. Current processing and packaging methods for catfish provide adequate quality protection for up to 3 months in frozen storage. Some studies have also shown that packaging techniques improve microbial quality of iced and refrigerated catfish. For example, fillets over-wrapped or vacuum-skin packaged still had good quality after 13 days of refrigerated storage at 4°C (Huang *et al.*, 1994). In another study, the shelf-life of catfish was extended for 19 days in a chill pack environment at –2.2°C (Reed *et al.*, 1983).

Psychrotrophic bacteria are capable of growing at ≤5°C and are known to grow on fillets that are stored on ice (chilling) at refrigerated temperatures. If the catfish is stored under aerobic conditions, psychrotrophic aerobes (e.g., *Acinetobacter, Flavobacterium, Moraxella, Pseudomonas* sp.) are the predominant spoilage bacteria. However, catfish stored under anaerobic conditions, the facultative anaerobic bacteria (e.g., *Alcaligenes* sp., *Brochothrix thermosphacta, Enterobacter* sp., *Enterococcus* sp., *Hafnia* sp., *Lactobacillus* sp., *Leuconostoc* sp., *Proteus* sp., *Serratia liquefaciens, Shewanella putrefaciens*) are the predominant bacteria.

Bacterial pathogens such as *A. hydrophila, Clostridium botulinum type E, E. coli, Listeria monocytogenes* and *Yersinia enterocolitica*, even though they have optimum and maximum temperatures for growth more characteristic of mesophiles, are considered to be psychrotrophic because their minimum growth temperature are below 5°C (Ray, 1996). Psychrotrophic bacteria can grow at low temperatures because a higher amount of unsaturated fatty acid maintains the plasma membrane in a liquid and

mobile state at temperatures below 5°C. This ensures that the membrane is biologically active and capable of absorbing nutrients at low temperatures (Garbutt, 1997).

Modified atmosphere packaging. The consumer demand for fresh catfish rather than frozen has prompted researchers to investigate alternative processing methods. MAP has been investigated to delay spoilage and extend the shelf-life of fresh catfish fillets while maintaining high quality. The method prevents the growth of aerobic bacteria in products that are either vacuum-packaged or flushed with 100% carbon dioxide (CO_2), 100% nitrogen (N_2), or a mixture of CO_2 and N_2. The antimicrobial effects of MAP can be produced by the changes in redox potential (Eh) and CO_2 concentrations. Aerobes and anaerobes have different Eh requirements for growth, while facultative anaerobes grow over a wide Eh range. The inhibitory effect of CO_2 on microbial growth include rapid cellular penetration and alteration in cell permeability, solubilization of CO_2 to carbonic acid within resulting in pH drop and subsequent lost of ATP, and interference of CO_2 with several enzymatic and biochemical pathways (Ray, 1996).

Some studies show that the MAP can be used in addition to ice or refrigeration. For example, catfish packaged in 80% CO_2 environment and stored at 2°C extended its shelf life by 3 weeks (Silva and White, 1994). In another study, holding catfish fillets strips at 0°C under MAP (90% CO_2) for 6 days and transferring to aerobic conditions for the remainder of storage, resulted in 8 days shelf-life extension (Silva *et al.*, 1993). Przybylski *et al.* (1989) report that using irradiation with or without elevated CO_2 concentrations reduced bacterial load and extended shelf-life of fresh iced catfish fillets to 20-30 days at 0-2°C.

Unfortunately, the great vulnerability of MAP foods from a safety standpoint is that with many modified atmospheres containing moderate to high levels of CO_2, the aerobic spoilage organisms (e.g., *Acinetobacter* sp., *Moraxella* sp., *Pseudomonas* sp.) which usually warn consumers of spoilage are inhibited, while the growth of anaerobic

pathogenic bacteria (e.g., *C. botulinum type E*) may be allowed or even stimulated. Nonsporeforming psychrotrophic pathogens, especially the facultative anaerobes (e.g., *L. monocytogenes, Y. enterocolitica*) can also multiply in fish products. In addition, some mesophilic facultative anaerobic pathogens (e.g., *E. coli, S. aureus, Salmonella* sp.) can grow if the MAP products are temperature abused during storage (Farber, 1991; Ray, 1996). This safety factor has limited the use of MAP in fresh catfish fillets.

Antimicrobial preservatives. Antimicrobial preservatives, or inhibitors, either present naturally or formed during processing or legally added as ingredients, are capable of killing microorganisms or controlling their growth in catfish. Some antimicrobial preservatives used to prolong the shelf-life of catfish are: acetic acid, lactic acid, lactic cultures, polyphosphates, potassium sorbate, propionic acid, sodium acetate, sodium ascorbate, sodium chloride, and sodium lactate. Fernandez *et al.* (1998a) state that antimicrobial properties vary with the quality and quantity of preservatives, and time of exposure. Organic acids, phosphates, spray washing, either individually or combined, have reduced the initial microbial counts on catfish, and have extended the shelf-life of the product. Table 1 shows the log reduction of aerobic plate counts on catfish fillets caused by different treatments.

Some studies show that organic acid dips or sprays, either individually or combined, are beneficial in controlling undesirable microorganisms on refrigerated catfish fillets. For example, a treatment containing 1% acetic acid and 1% lactic acid for 30 s at 4^{o}C suppressed growth of aerobic microorganisms on catfish fillets for 4 days and extended shelf-life to 10 days (Marshall and Kim, 1996). In another study, the combination of a 2.5% lactic culture and 3% lactic acid dip (1 min) extended the refrigerated shelf-life of catfish fillets for an additional 3 days at 4 or 10^{o}C and showed that it was effective in suppressing Gram-negative bacteria. This study suggests that lactic acid producing bacterial cultures would have potential use in suppressing the growth of spoilage organisms by means of pH reduction and bacteriocins effects,

whereas lactic acid has antimicrobial properties due to the undissociated acid molecule as well as its pH effect (Kim *et al.*, 1995a). Fernandez *et al.* (1998a) found that spray washing improved the microbial quality of catfish fillets due to both washing as well as the sublethal and lethal effect of weak organic acids (1% lactic acid or 1% propionic acid) on the indigenous microflora of fillets.

Table 1. Log reduction on catfish fillets caused by different treatments

Treatment conditions	Log reduction[a]	Author
Spray washing with water	0.5	Fernandez *et al.*, 1998a
Spray washing with 1% lactic or 1% propionic acid	1.0	Fernandez *et al.*, 1998a
In 1% sodium acetate or combination of 0.75% sodium acetate and 4% monopotassium; tumbling for 15 min at 18 rpm	0.6	Kim *et al.*, 1995b
In 10% trisodium phosphate for 5 min at 5°C	1.0	Marshall and Jindal, 1997
In 1% acetic acid and 1% lactic acid for 30 s	0.5	Marshall and Kim, 1996
In 2% sodium lactate; tumbling under vacuum for 15 min	1.0	Williams *et al.*, 1995

[a] Log values of aerobic plate counts measured in CFU/g.

In addition, Kim and Hearnsberger (1994) report that the combination of 0.50% sodium acetate, 0.25% potassium sorbate with 2.50% lactic acid culture completely inhibited growth of Gram-negative bacteria for at least 6 days at 4°C. This study showed that sodium acetate, potassium sorbate, and metabolites produced by lactic acid bacteria had an antimicrobial effect on food spoilage organisms. Also, combining 0.75% sodium acetate and 0.40% monopotassium phosphate or 1.00% sodium acetate

alone inhibited the growth of aerobic microorganisms of refrigerated (4°C) catfish fillets and extended their microbiological shelf-life to 12 days (Kim *et al.*, 1995b). Marshall and Jindal (1997) demonstrated that 10% trisodium phosphate solution dip for 5 min at 5°C was effective in reducing microbial numbers on the surface of catfish frames (the carcasses remaining after skinless boneless fillets are removed), resulting in a subsequent shelf-life extension of 3 days. Williams *et al.* (1995) report that 2% sodium lactate could be employed as an antimicrobial agent and as an antioxidant in fresh bullhead catfish fillets stored at 1°C. Their studies extended the shelf-life of bullhead catfish to 4-7 days.

The Grovac processing method. The Grovac process has been developed for enhancing the flavor and shelf-life of fresh catfish fillets. This process uses partial vacuum on catfish fillets submerged in a processing solution of ascorbic acid (AA) and sodium chloride (NaCl). The use of vacuum in combination with the processing solution has a profound effect on the growth and viability of microbial cells. For example, AA acts as a pH-reducing solute when it is added to the solution. When the pH is reduced below the lower limit for growth of a microbial species, not only do the cells stop growing, but they also lose viability, the rate of which depends upon the extent of pH reduction (Ray, 1996). This is more apparent when a weak organic acid such as AA is used because of its high pK value (4.0) (Gregory, 1996). A high pK value means more of the molecules are in the undissociated form which are subsequently capable to entering the cell; where they dissociate to generate H^+ in the cytoplasm. The high H^+ concentration has an adverse effect on the proton gradient between the inside and the outside of the cells. To overcome this problem, the cells pump out the protons by expending energy (ATP). This represents a large amount of energy that cells are not able to replenish. In addition, the low pH can act on the cellular enzyme and adversely affect their structural (by interfering with the ionic bonds) and functional integrity (Lyon, 1998; Ray, 1996). In addition to its antimicrobial properties,

AA is an antioxidant reducing the potential for flavor and color changes. The antioxidative role of AA include free radical scavenging and oxygen scanvenging. Their acidic and reducing properties are contributed by the 2,3-enediol moiety (Gregory, 1996).

Salt in the Grovac process is not employed as a preservative because of the low concentrations (<1% w/v) used. However, it seems to be unique in that it has the ability to enhance desirable flavor by suppressing unpalatable flavors such as bitterness and sourness. This effect is believed to be due to the selective filtration of flavors by sodium ions which are released when salt is dissolved (Niman, 1997). Luck and Jager (1997) state that the concentrations of salt required for flavor enhancement are generally much lower than those needed for preservation purposes. In addition, low concentrations of salt are used to reduce handling stress in channel catfish when they are transported live to the plant. The addition of up to 8 g/L (0.8%) salt solutions can minimize the loss of blood salt (0.9%) during transport. Thus salt is beneficial for calming excitable fish that might injure themselves in transit. Sodium and potassium are critical for the normal function of heart, nerve and muscle of catfish (Wurts, 1995).

According to Groves (1996), the hypotonic solution of AA and NaCl also enhances osmosis of compounds within the cellular structure which contributes significantly to the dilution and extraction of the geosmin content and off-odors. Besides the processing solution, vacuum tumbling also contributes to bacterial lysis of bacteria associated with catfish fillets. In foods under vacuum, the low oxygen tension inside the pack inhibit both chemical oxidation and microbial (proteolytic or lipolytic) activity (Church, 1998).

There is evidence that some control methods, individually or in combinations, discussed earlier can extend the shelf-life of refrigerated catfish fillets. However, bacterial hazards associated with any process must be addressed. The Grovac process has not been evaluated on its effect on pathogenic bacteria associated with fresh catfish

fillets. One must consider the overall safety of each product and evaluate whether the process increases or decreases pathogens associated with this product. For example, Fernandez *et al.* (1998b) observed that both individually and simultaneously inoculated psychrotrophic pathogens *L. monocytogenes* and *A. hydrophila* could grow on aquacultured catfish fillets stored in plastic bags at refrigeration temperatures (4°C). In another inoculation study, *L. monocytogenes* did not grow on overwrapped packaged catfish fillets stored at 4°C while *A. hydrophila* grew at a rate similar to the naturally occurring psychrotrophic bacteria (Leung *et al.*, 1992a).

MATERIALS AND METHODS

Isolation

Fish source. WC and CF were obtained from Tony's Live Catfish & Seafood (Baton Rouge, LA) during the spring and the summer of 1998, respectively. WC arrived at the processing plant alive in aerated tanks via hauling trucks. The WC were held in raceways with aeration until the plant was ready to process them. WC were electrically shock which stunned them before they were manual headed, gutted, skinned, and filleted. CF were not washed before they were packaged, thus giving them a bloody appearance. WC or CF were bagged in polyethylene bags, transported on ice to the Louisiana Department of Agriculture & Forestry (LDAF)/Louisiana State University (LSU) Rapid Microbial Detection Laboratory, stored at refrigeration temperature (4°C) and subjected to microbiological analysis within 24 h.

Media preparation. Selective and nonselective culture media were used to isolate bacteria from both WC and CF. Nonselective media tryptic soy agar (TSA; Difco, Detroit, MI) was used to isolate endogenous bacterial flora as recommended by Austin and Austin (1989) and Inglis *et al.* (1993). TSA (Difco) was prepared as described by the manufacturer for the isolation and cultivation of aerobic and facultative anaerobic bacteria from samples.

The isolation of pathogenic bacteria from WC and CF, in the presence of a high abundance of nonpathogenic microflora, required the use of selective, differential, or both selective and differential media such as Cefsulodin Irgasan Novobiocin (CIN; BBL, Cockeysville, MD) agar, Mannitol Yolk Polymyxin (MYP; Difco, Detroit, MI) agar, Modified Cellobiose Polymyxin Colistin (MCPC; Difco, Detroit, MI) agar, Modified Oxford (MOX; Oxoid, Columbia, MD) agar, Pseudomonas F (PF; Acumedia, Baltimore, MD) agar, Starch Ampicillin (SA; Difco, Detroit, MI) agar, Sulfite Polymyxin Sulfadiazin (SPS; Difco, Detroit, MI) agar, Thiosulfate Citrate Bile Salt Sucrose (TCBS; Difco, Detroit, MI) agar, Tryptose Sulfite Cycloserine (TSC; Oxoid,

Columbia, MD) agar, Violet Red Bile (VRB; Difco, Detroit, MI) agar, and Xylose Lysine Deoxycholate (XLD; Difco, Detroit, MI) agar. In addition, Total Coliform & *E. coli* SimPlate (IDEXX, Westbrook, ME) was used for the detection and quantification of the total coliform and *E. coli* in the samples.

The difference among selective, differential, and both selective and differential media is determined by the ingredients of each medium. Selective media contained ingredients that inhibited the growth of certain organisms but allow the growth of other bacteria. Differential media contained ingredients that were changed as a result of microbial metabolism (Atlas, 1997; Inglis *et al.*, 1993; Koneman *et al.*, 1992; Lyon, 1998). The use of selective and nonselective media for the isolation or/and differentiation of microorganisms from WC and CF is given in Table 2.

The ingredients that make up these types of culture media and their purpose for the cultivation of microorganisms were: (1) Protein hydrolysates such as peptones, meat infusion, tryptones and casein to provide the carbon and nitrogen needed for bacterial metabolism; (2) Carbohydrates such as lactose, sucrose, maltose, dextrose and xylose to provide a ready source of carbon for energy and to serve as substrates in biochemical reactions for identification of unknown organisms; (3) Buffers such as monosodium and disodium or potassium phosphates to provide a stable pH for optimal growth of microorganisms; (4) Enrichments such as serum, vitamin supplements and yeast's extracts to recover fastidious organisms; (5) Inhibitors to suppress the growth of certain undesired bacterial species while permitting the cultivation of the target microorganism of interest, such as dyes (e.g., crystal violet, methylene blue, brilliant green and eosin), heavy metals (e.g., bismuth), toxic chemicals (e.g., bile salts, selenite, tetrathionate, tellurite, azide, phenylethanol, sodium lauryl sulfate, high sodium chloride concentrations, sodium deoxycholate, and citrate), antibiotics (e.g., ampicillin, chloramphenicol, colistin, cycloserine, cycloheximide, gentamicin, kanamycin, polymyxin B, sulfadiezine, and vancomycin); (6) pH indicators such as methylene blue,

neutral red, phenol red and bromocresol purple to measure pH shifts in test media resulting from bacterial metabolism of given substrates; and (7) Other compounds such as ferric and ferrous ions for the detection of hydrogen sulfide, and sodium thiosulfate to provide a source of sulfur (Atlas, 1997; Koneman *et al.*, 1992; Lyon, 1998).

Table 2. Use of selective and nonselective media for the isolation or/and differentiation of microorganisms from whole catfish (WC) and catfish fillets (CF)

Medium	Use
CIN (BBL) agar	*Yersinia enterocolitica*
MYP (Difco) agar	*Bacillus cereus*
MCPC (Difco) agar	*Vibrio* sp.
MOX (Oxoid) agar	*Listeria monocytogenes*
PF (Acumedia) agar	*Pseudomonas aeruginosa*
SimPlate (IDEXX)	Total coliform and *Escherichia coli*
SA (Difco) agar	*Aeromonas hydrophila*
SPS (Difco) agar	*Clostridium* sp.
TCBS (Difco) agar	*Vibrio* sp.
TSA (Difco) agar	Fastidious microorganisms
TSC (Oxoid) agar	*Clostridium* sp.
VRB (Difco) agar	Coliforms
XLD (Difco) agar	*Salmonella* and *Shigella*

Catfish sample preparation. A representative 25-g sample of WC was obtained by using the quadrant diminutive sampling method (Lyon, 1998). This method involves aseptically excising with a sterile knife three-25 g sub-samples from the lateral line (gill, center, and posterior) of one side and two-25 g sub-samples from the other side (lateral anterior and posterior). In the case of CF, a 25-g sample was obtained from

the lateral line of both extremes and the center of the fillet. Then the sample either WC or CF, was placed in a sterile stomacher bag (VWR, West Chester, PA) with 225 ml of Buffered Peptone Water (BPW; Difco, Detroit, MI, model No. STO-400), and homogenized (Tekmar, Cincinnati, OI) for 2 min at room temperature.

The homogenized sample (10^{-1}) dilution was serially diluted using 9 ml BPW (Difco) dilution blanks. Each dilution was plated on medium in duplicate using the spread plate technique (Lyon, 1998). Dilutions plated onto TSA (Difco) were incubated at 37°C for 24 h under normal aerobic conditions. Incubation temperature and the gaseous atmosphere were selected based on the organisms oxygen requirements. For example, samples plated on MYP (Difco) and CIN (BBL) agar were incubated at 30°C for the isolation of *Bacillus cereus* and *Yersinia* sp., respectively. Samples plated on MCPC (Difco) were incubated at 40°C for the isolation of *Vibrio* sp. Samples plated on SPS (Difco) agar and TSC (Oxoid) agar, and TSA (Difco) were incubated at 37°C for 24 h in anaerobic conditions using the GasPak system (BBL, Baltimore, MD) for the isolation of *Clostridium perfringens* and anaerobic bacteria, respectively. After incubation, the number of colonies on countable plates with between 30-300 colonies were counted and recorded.

Anaerobic culture technique. The GasPak system (BBL, Baltimore, MD) was used to generate hydrogen and carbon dioxide for the primary isolation of anaerobic bacteria. Hydrogen was generated from sodium borohydride tablet following the addition of water into the GasPack Plus (BBL) envelope. Hydrogen combined with the oxygen in the jar in the presence of the palladium catalyst to form water. In addition, 4 to 10% carbon dioxide was generated from a sodium bicarbonate plus citric acid tablet. The carbon dioxide was provided to stimulate the growth of anaerobes that require CO_2-enriched atmosphere for growth. An anaerobic indicator (Oxoid, Columbia, MD) was used to verify anaerobic conditions in the anaerobic jar during the incubation period.

Enumeration. Aerobic plate count (APC) provided an assessment of the microbial quality of WC and CF. APC was determined by counting the colony forming units (CFU) after incubation of plates. APC (CFU/g) was calculated as follows: $N = \Sigma C / [(1 \times n_1) + (0.1 \times n_2)] \times (d)$. Where N = Number of colonies per g of product, ΣC = Sum of all colonies on all plates counted, n_1 = Number of plates in first dilution counted, n_2 = Number of plates in second dilution counted, and d = Dilution from which the first counts were obtained (Peeler and Maturin, 1992).

In addition, SimPlate (IDEXX) tests were done by transferring 1 ml of a 10^{-1} dilution into 9 ml of a pre-measured media. The sample was mixed and poured onto a SimPlate devise and incubated at 37°C for 18-24 h. Total coliforms and *E. coli* count were determined by counting the number of positive wells and referring to the most probable number (MPN) table. Colored wells indicated the presence of coliforms and colored wells that fluoresce indicated the presence of *E. coli*.

SimPlate (IDEXX) test equates the presence of total coliforms and *E. coli* to the presence of β-galactosidase and β-glucuronidase activity, respectively. When *E. coli* is present, its enzyme glucuronidase releases 4-methylumbelliferone, which is fluorescent, from the substrate 4-methylumbelliferyl-D-glucuronide added to the medium (Lyon, 1998). To detect the substance, the plates were illuminated with long-wave ultraviolet light using a UV lamp (Mineralight Lamp, Ultraviolet Products, San Gabriel, CA, Model UVGL-48).

Statistical analyses. All data was analyzed by a statistical analysis system (SAS; SAS Institute Inc., Cary, NC; Edition 6.12; 1996) computer program. Statistical methods included paired dependent t-test, analysis of variance (ANOVA), general linear model (GLM) procedure, and Tukey's Studentized Range Test (Ott, 1993). Significance was based on a probability level of 0.05 ($p<0.05$).

Identification of Bacterial Isolates

Preliminary identification. Preliminary identification of bacterial isolates from WC and CF was based on phenotypic tests such as colony characteristics, Gram-stain reaction, oxidase test, catalase production, and coagulase (Koneman *et al.*, 1992; Lyon, 1998, Yu and Washington, 1985). Colony characteristics such as form, elevation, margin, density, color, and size were noted after incubation of plates on both nonselective and selective media. Colonies from both nonselective and selective media were streaked onto TSA (Difco) and incubated at 37°C for 18 h. Individual isolates were further identified by using rapid microbial identification as described later.

The Gram-stain reaction was performed to differentiate Gram-positive from Gram-negative organisms on the basis of differences in cell wall composition and thus differences in cellular permeability to a solvent such as ethanol. For this reaction, heat-fixed smears were stained for 1 min with crystal violet, washed in tap water, covered with Gram's iodine for 1 min, re-washed, decolorized for 30 s in 95% ethanol, re-washed, and counterstained for 10-30 s with safranin solution (Poelma and Bryce, 1992). Gram-positive bacteria stained blue or purple while Gram-negative bacteria stained red or pink. Crystal violet, Gram's iodine and safranin solutions (Difco), and ethanol were used to perform this reaction. Bacterial morphology such as rods, coccus and vibrio, and arrangements of bacterial cells were determined microscopically under oil immersion.

The oxidase test was performed on all Gram-negative bacteria to determine the presence or absence of the enzyme cytochrome c. This enzyme is produced by certain bacteria (e.g., *Aeromonas*, *Flavobacterium*, *Vibrio*) that can use oxygen as a final electron acceptor in their energy metabolism. Organisms containing cytochrome c were oxidase-positive. In a positive reaction, the enzyme combined with N, N-dimethyl-p-phenylenediamine oxalate and α-napthol to form the dye indophenol blue. Inversely, organisms lacking cytochrome c (e.g., *E. coli*) as part of their respiratory

chain did not oxidize the reagents, leaving it colorless, and they were considered oxidase-negative. Oxidase BR64 (Oxoid, Columbia, MD) sticks were used to perform the oxidase test as described by the manufacturer.

The catalase test was performed on all Gram-positive bacteria to separate staphylococci that are catalase-positive from streptococci that are catalase-negative. Catalase is an enzyme used by living cells to remove the toxic hydrogen peroxide. Bacteria thereby protect themselves from the lethal effect of hydrogen peroxide, which is accumulated as an end product of aerobic carbohydrate metabolism. Catalase-positive bacteria (e.g., aerobes, *S. aureus*) decomposed hydrogen peroxide to water and oxygen (gas bubbles). Also, catalytic decomposition of hydrogen peroxide involved the reduction of trivalent iron (Fe^{3+}) in catalase (hemoprotein) by hydrogen peroxide to its reduced form (Fe^{2+}) and the reoxidation of the latter by oxygen. A 3% hydrogen peroxide (EM, Gibbstown, NJ) solution was used to perform this test.

The coagulase test was performed to separate *S. aureus*, which possesses coagulase activity from coagulase-negative *Staphylococcus* sp. *S. aureus* has the ability to produce free and bound coagulase (or clumping factor). The Staphylase Test Kit DR595 (Oxoid, Columbia, MD) detects the presence of the clumping factor by clumping fibrinogen-CF-sensitized sheep red blood cells.

Final Identification. Preliminary identification of bacterial isolates from WC and CF was followed by further identification by using the Vitek Automated Identification System (bioMérieux Vitek, Inc., Hazelwood, MO). Vitek™ gram-negative identification (GNI+) and gram-positive identification (GPI) cards were used for the identification of Gram-negative and Gram-positive bacteria, respectively. There are thirty wells in the GNI+ card which contained twenty-eight biochemical broths, one negative control broth and one growth control broth. The GNI+ card is designed to analyze both conventional and non-conventional biochemical tests for identification of Gram-negative bacteria.

Some conventional biochemical tests are based on the ability of bacteria to ferment carbohydrates or/and degradate amino acids. In a fermentation, a given carbohydrate may be fermented to a number of different end products (e.g., alcohols, organic acids, carbon dioxide, hydrogen, water) depending upon the microorganism involved. Therefore, these end products are characteristic of the particular bacterium and can be used as identifications tools. For example, the fermentation of glucose produces acids and lowers the pH. If a pH indicator such as phenol red or bromcresol purple is included in the medium, the acid production changes the medium from its original color to yellow (Lyon, 1998).

In the degradation of amino acids by certain bacteria, the main reactions involved are decarboxylation and deamination. As a consequence of these reactions, many products such as carbon dioxide, hydrogen, ammonia, hydrogen sulfide, organic acids, alcohols, amines, diamines, mercaptans and organic disulfides may be formed. For example, decarboxylation of lysine or ornithine results in the production of an amine and carbon dioxide. This amine causes an increase in the pH of the medium and a change of its color from yellow to blue (Lyon, 1998). A summary of the biochemical tests and their principles performed by Vitek^{TM} GNI+ card are given in Table 3.

The Gram-stain reaction, oxidase test and bacterial suspension were prepared prior to filling the GNI+ card. Bacterial preparation involved preparing a suspension of the organism in sterile saline (0.4% w/v). All cell suspensions were standardized to a specific absorbance using the Vitek colorimeter as described by the manufacturer (bioMérieux). Cell suspensions were attached to specific cards via a transfer tube, placed in the filling module of the instrument, and inoculated by a vacuum/release method. The cards were then placed in the reader/incubator module of the Vitek, where it was optically scanned and read periodically.

Table 3. Biochemical tests and principles of the Vitek™ GNI+ card

Medium	Principle
DP-300	Glucose fermentation in the presence of specific inhibitors.
Glucose (oxidative)	Aerobic acid production from oxidation of the carbohydrate.
Growth control	Support the growth of most Gram-negative bacteria at 35°C.
Acetamide	Change in pH occurs due to utilization of acetamide.
Esculin	Hydrolysis of esculin to esculetin which reacts with iron compounds forming a black precipitate.
Plant indican	A unique compound is split from a β-D-glucoside group. The structure formed, in the presence of oxygen, results in the black precipitate indigo blue.
Urea	Urease release ammonia from urea; free ammonia causes the pH to increase and changes the indicator from green to blue.
Citrate	Citrate utilization results in a pH increase and change the indicator from yellow to blue.
Malonate	Utilization of malonate as the source of carbon causes a rise in pH and changes the indicator from yellow to dark green to blue.
Tryptophan	Tryptophan diaminase forms indolepyruvic acid which reacts with iron compounds resulting in a brown color.
Polymyxin B	Growth in the presence of polymyxin B.
Lactose	Aerobic acid production from oxidation of the carbohydrate.
Maltose	Same as lactose.
Mannitol	Same as lactose.
Xylose	Same as lactose.

(TABLE continued)

Raffinose	Utilization of the carbohydrate results in acid formation with a consequent pH drop.
Sorbitol	Same as raffinose.
Sucrose	Same as raffinose.
Inositol	Same as raffinose.
Adonitol	Same as raffinose.
p-Coumaric	Glucose fermentation in the presence of specific inhibitors.
Hydrogen sulfide	Hydrogen sulfide is produced from thiosulfate which reacts with iron salts to produce a black precipitate.
O-Nitrophenyl-β-D-galacto-pyranoside (ONPG)	Hydrolysis of ONPG by β-D-galactosidase releases ortho-nitrophenol from β-D-galacto-pyranoside.
Rhamnose	Utilization of the carbohydrate results in acid formation with a consequent pH drop.
L-Arabinose	Same as rhamnose.
Glucose (fermentative)	Same as rhamnose.
Arginine	Arginine dihydrolase transforms arginine into ornithine, ammonia and carbon dioxide. This causes an increased pH and a change in the indicator from yellow to blue.
Lysine	Lysine decarboxylase transforms lysine into a basic amine, cadaverine. This amine causes an increased pH and a change from yellow to blue.
Ornithine	Ornithine decarboxylase transforms ornithine into a basic amine, putrescine. This amine causes an increased pH and a change from yellow to blue.
Decarboxylase	Control.

The GPI card is also designed to analyze a battery of conventional and non-conventional biochemical tests for identification of Gram-positive bacteria. A summary of the biochemical tests and their principles performed by the Vitek^{TM} GPI card are given in Table 4.

Table 4. Biochemical tests and principles of the Vitek^{TM} GPI card

Medium	Principle
Peptone Base	Growth indicated by acid production. pH drop causes change in indicator.
Bacitracin	Inhibits growth by preventing cell wall synthesis. The carbohydrates in the medium not utilized and therefore pH does not drop.
Optochin	Inhibits growth, causing lysis of cell membrane.
6% Sodium Chloride	Tolerance to high NaCl concentration indicated by acid production and indicator change.
10% Bile	Tolerance to high levels of bile indicated by acid production and indicator change.
Esculin	Esculin hydrolized to esculetin which reacts with ferric citrate to form a brown-black complex.
Arginine	Arginine dihydrolase transforms arginine into ornithine, ammonia and carbon dioxide. This causes an increased pH and a change in the indicator from yellow to blue.
Urea	Urease releases ammonia from urea; free ammonia causes the pH to increase and changes the indicator from yellow to blue.
Tetrazolium red	Upon reduction the tetrazolium salt forms a red precipitate.
Novobiocin	Inhibits synthesis of DNA and teichoic acid at the membrane.

(TABLE continued)

Hemicellulase	Utilization of carbohydrate results in acid formation. The pH drop causes an indicator shift from clear or light blue to dark blue.
Dextrose	Same as hemicellulase.
Lactose	Same as hemicellulase.
Mannitol	Same as hemicellulase.
Raffinose	Same as hemicellulase.
Salicin	Same as hemicellulase.
Sorbitol	Same as hemicellulase.
Sucrose	Same as hemicellulase.
Trehalose	Same as hemicellulase.
Arabinose	Same as hemicellulase.
Pyruvic acid	Same as hemicellulase.
Pullulan	Same as hemicellulase.
Inulin	Same as hemicellulase.
Melibiose	Same as hemicellulase.
Melezitose	Same as hemicellulase.
Cellobiose	Same as hemicellulase.
Ribose	Same as hemicellulase.
Xylose	Same as hemicellulase.
Decarboxylase	Control

The Vitek computer determined whether each well was positive or negative by measuring the light attenuation with an optical scanner. Fermentative Gram-negative organisms usually require 2-8 h for identification while nonfermentative Gram-negative

bacteria require incubation periods of 10-13 h. Gram-positive organisms usually required 10 to 13 h incubation periods for identification. In addition to the Gram-stain reaction, the GPI card is externally marked in designated areas for the presence or absence of catalase and coagulase activity. Final identification of the unknown Gram-positive isolate is determined by comparing the positive and negative biochemical pattern of the wells and the external enzyme marks with the computer database.

Storage of bacterial isolates. Some nonpathogenic and pathogenic bacteria isolated from WC and CF were stored for later inoculation studies. A bacterial suspension was prepared by inoculation of trypticase soy broth (TSB; BBL, Cockeysville, MD) with a single bacterial colony and incubation at 37°C for 18 h. An aliquot of 0.5 ml of the bacterial culture was transferred to a sterile vial containing 0.5 ml of sterile glycerol, mixed thoroughly by vortexing, and stored at -80°C (Maniatis *et al.*, 1982).

Evaluation of the Grovac Processing Method

A series of experiments were conducted to evaluate the reduction of endogenous microflora of CF using the Grovac processing method. This process involved the use of an equipment composed of a vacuum tumbler (Lyco, Janesville, WI, Model LT-40) with a vacuum pump (Ritchie, Bloomington, MN, Model No. 93000) and a cylindrical drum with perforated paddles.

Experiment 1. This experiment was conducted to evaluate the individual and combination effects of AA plus NaCl and vacuum on the reduction of microbial load in CF using the Grovac process. Duplicate experimental trials consisted of the following treatments: (A) Water + vacuum (4 in. Hg to keep the lid closed); (B) water + 0.4% AA + 0.4% NaCl + vacuum (4 in. Hg to keep the lid closed); (C) water + vacuum (28 in. Hg); and (D) water + 0.4% AA + 0.4% NaCl + vacuum (28 in. Hg). The processing solution for treatment B or D was prepared by dissolving AA (0.4% w/v) and NaCl

(0.4% w/v) in 2 L of autoclaved tap water at ambient temperature. Two liters of autoclaved tap water at ambient temperature were used for treatment A and C.

A total of 40 CF were obtained from Tony's Live Catfish & Seafood (Baton Rouge, LA) less than 1 h postmortem, bagged in polyethylene bags, transported to the LDAF/LSU Rapid Microbial Detection Laboratory on ice, stored in a cold room at 4°C, and processed within 12 h. Ten fillets at random were assigned to each treatment. Before processing, each fillet was split lengthwise and marked with small cuts for identification purposes. One half of the fish fillet was considered the control while the other one was subjected to a given treatment. Control samples were packaged in sterile bags (Whirl-Pak; Nasco, Madison, WI) and stored at 4°C until microbiological analyses were done.

Five half CF were used per treatment replication. The CF were placed in the vacuum tumbler at ambient temperature. CF and processing solution were set up for all treatments by using 400 g of catfish to 2 L of processing solution. Either sterile water or the sterile processing solution was added to the tumbler. Air from the tumbler was withdrawn to create a partial vacuum. The tumbler was rotated at 8 rpm for 8 min to expose the CF either to water or the processing solution. During tumbling, CF were held out of water or processing solution by the paddles. It was during this time that the fillets were subjected to partial vacuum pressure. Treated samples were aseptically removed from the tumbler, drained for 2 min, and placed in sterile bags (Whirl-Pak; Nasco). The pH of water or processing solution was measured before and after each treatment by using a pH meter (Orion, Boston, MA, Model 410A).

After processing, three half fillets out of five were taken randomly for microbiological analyses. Three fillets were selected randomly to avoid bias. A 25 g sample was taken aseptically from each half fillet by using the quadrant diminutive sampling method (Lyon, 1998) and placed in a sterile stomacher bag (VWR). Then the sample was homogenized with 225 ml of BPW (Difco) for 2 min at room temperature.

From this 10^{-1} homogenate, serial decimal dilutions were prepared in 9 ml BPW (Difco) dilution blanks. Aliquots of 0.1 ml of each dilution were plated on TSA (Difco) in triplicate using the spread plate technique (Lyon, 1998). The plates were incubated at 37°C for 24 h under normal aerobic conditions. After incubation, the number of colonies on countable plates with between 30-300 colonies were counted and recorded. APC count was determined as described earlier. Mean values were reported of each sample. Data derived from plate counts were transformed into logarithms (log CFU/g). Log reduction for each fillet was calculated as log initial (control) minus log final (treated). The mean reduction between log initial and log final means from each treatment was subjected to a paired dependent t-test. In addition, log reduction means of all treatments were subjected to the ANOVA.

Experiment 2. This experiment was conducted to evaluate the effectiveness of 3 levels of AA (0.4, 0.8 and 1.2%) and 3 levels of NaCl (0.2, 0.4 and 0.6%) on the reduction of microbial loads in CF using the Grovac process. A 3 x 3 factorial design with two replications was used. Therefore, there were a total of 9 possible treatment combinations of AA and NaCl. Duplicate experimental trials consisted of the following treatments: (A) 0.4% AA + 0.2% NaCl; (B) 0.4% AA + 0.4% NaCl; (C) 0.4% AA + 0.6% NaCl; (D) 0.8% AA + 0.2% NaCl; (E) 0.8% AA + 0.4% NaCl; (F) 0.8% AA + 0.6% NaCl; (G) 1.2% AA + 0.2% NaCl; (H) 1.2% AA + 0.4% NaCl; (I) 1.2% AA + 0.6% NaCl.

A total of 54 CF were obtained from Tony's Live Catfish & Seafood (Baton Rouge, LA) less than 1 h postmortem, bagged in polyethylene bags, transported to the LDAF/LSU Rapid Microbial Detection Laboratory on ice, stored in a cold room at 4°C, and processed within 12 h. Six fillets at random were assigned to each treatment. Before processing, each fillet was split lengthwise and marked with small cuts for identification purposes. One half of the fish fillet was considered the control while the other one was subjected to a given treatment. Control samples were packaged in sterile

bags (Whirl-Pak; Nasco, Madison, WI) and stored at 4°C until microbiological analyses were done. Three half CF were used per treatment replication. The CF were placed in the vacuum tumbler at ambient temperature. CF and processing solution were set up for all treatments by using 400 g of catfish to 2 L of processing solution. Grovac experimental conditions were: Vacuum = 28 in. Hg; tumbling time = 8 min; and rotation = 8 rpm. The Grovac process was conducted as described earlier. After processing, treated samples were aseptically removed from the tumbler, drained for 2 min, and placed in sterile bags (Whirl-Pak; Nasco). The pH of water or processing solution was measured before and after each treatment by using a pH meter (Orion, Boston, MA, Model 410A).

Microbiological analysis, APC determination and log transformation of the data were done as described earlier. APC reduction (%) on CF was calculated as follows: (Initial CFU/g - Final CFU/g) (100) / Initial CFU/g (Ray, 1996). Log reductions means of all treatments were analyzed by using the GLM procedure and Tukey's Studentized Range Test of the SAS (SAS Institute Inc., Cary, NC; Edition 6.12; 1996) computer program. The interactions between AA and NaCl was also tested.

Experiment 3. An inoculation study was conducted to evaluate the Grovac process for its ability to reduce *A. hydrophila* inoculated on CF, and to follow the survival and/or growth of *A. hydrophila* on refrigerated CF at 4°C during storage. Before the inoculation, an experiment was performed to determine the growth of *A. hydrophila* isolated from CF. *A. hydrophila* which had been previously isolated from CF and stored at -80°C was selected for this experiment. Viable bacteria of this culture were recovered by scratching the surface of the frozen stock with a sterile platinum loop and streaked on a TSA (Difco) plate. The plate was incubated at 37°C for 18 h. A cell suspension of *A. hydrophila* was prepared by inoculating a fresh colony in 100 ml of TSB (BBL) at 37°C on an orbital shaker (Forma Scientific, Maujetta, OI, Model 4535) at 250 rpm. Aeration of the culture was achieved by attaching the 500 ml flask to the

shaker platform. Experiment conditions take into account that the rate of growth is dependent on the medium, volume of medium, size of flask, temperature, and the degree of aeration (Lyon, 1998; Maniatis *et al.*, 1982).

The rate of growth of *A. hydrophila* was monitored by withdrawing aliquots of 2 ml of the bacterial suspension every 30 min and reading the optical density (OD; Milton Roy, Model Spectronic 21D) at a wavelength of 600 nm. In addition to these aliquots, other aliquots of 1 ml were withdrawn to prepare serial decimal dilutions in 9 ml BPW (Difco) dilution blanks. Each dilution was plated on TSA (Difco) in duplicate. *A. hydrophila* counts were determined as described earlier. A growth curve that relates the number of viable bacteria (CFU/ml) to the OD was plotted and used in further experiments.

For the inoculation, a cell suspension of *A. hydrophila* was prepared as described previously. A culture suspension of approximately 10^9 CFU/ml (OD_{600} = 0.490) was prepared and 1 ml of the suspension was centrifuged at 1,400 rpm for 5 min in a centrifuge (Brinkmann Instruments,Westbury, NY, Model 5415C) to pellet the bacterial cells. The cells were then washed twice with Buffer Phosphate Saline (BPS; pH 7.4). The cells were resuspended in 1000 ml of sterile BPS to give a final concentration of 10^6 CFU/ml. CF were inoculated by submerging them in the cell suspension as described later.

Six CF (average weight approximately 400 g per fillet) were obtained from Tony's Live Catfish & Seafood (Baton Rouge, LA) less than 1 h postmortem, bagged in polyethylene bag, transported to the LDAF/LSU Rapid Microbial Detection Laboratory on ice, stored in a cold room at 4°C, and used within 12 h. Each CF was split lengthwise along the spinal column. One piece of the fillet was assigned to the uninoculated group (control) and the other one to the inoculated group. In turn, each piece was trimmed to a weight of 25 g and marked with small cuts for identification purposes, prior inoculation. Samples of the inoculated group were submerged into the

cell suspension of *A. hydrophila* for 15 min and placed into sterile bags (Whirl-Pak; Nasco). Uninoculated and inoculated samples were subjected to the Grovac processing method under the following experimental conditions: Processing solution of AA 0.8% (w/v) and NaCl 0.6% (w/v) = 2 L; vacuum = 28 in. Hg; tumbling time = 8 min; and rotation = 8 rpm. The processing solution was selected from experiment 2 because of its ability to reduce microbial counts approximately 2 log CFU/g (APC reduction 99%). CF and processing solution were set up for uninoculated and inoculated samples by using 400 g of catfish to 2 L of processing solution.

The survival and/or growth of *A. hydrophila* on refrigerated CF at 4°C was evaluated during storage. Samples were stored at 4°C in a cooling room for up to 7-days. On days 0, 3, 5, and 7, samples of uninoculated and inoculated fillets were analyzed for *A. hydrophila* and APC counts on *A. hydrophila* medium (Oxoid) and TSA (Difco), respectively, as described earlier. CFU/g on CF were calculated as described earlier. The APC and *A. hydrophila* means of uninoculated and inoculated CF at 0, 3, 5 and 7 days were analyzed by using paired dependent t-tests. In addition, APC and *A. hydrophila* means of uninoculated and inoculated CF during storage time were analyzed by using the ANOVA and Tukey's Studentized Range Test of the SAS (SAS Institute Inc., Cary, NC; Edition 6.12; 1996) computer program.

RESULTS AND DISCUSSION

Isolation and Identification of Bacteria from WC and CF

Number and types of bacteria. The principles and procedures (e.g., aseptic sampling technique, culture media, biochemical characterization) followed for the isolation and identification of bacteria in WC and CF were essentially the same as those used in mammalian bacteriology. A total of fifty-one bacterial isolates were isolated and identified from WC and CF using selective and nonselective culture media, and the Vitek^{TM} System (bioMérieux; Table 5). From this total of bacterial isolates, twenty types of bacteria were associated with WC. The low number of bacterial isolates associated with WC could be due to the selectivity of the skin against some bacteria (Austin and Austin, 1989). The microflora isolated from WC is representative of bacteria normally found in fresh and sea water (e.g., *Acinetobacter* sp., *A. hydrophila, Flavobacterium, E. coli, Vibrio* sp.); surfaces of freshwater and marine fishes (e.g., *Acinetobacter* sp., *Aeromonas* sp.); and intestinal microflora in normal channel catfish (e.g., *Acinetobacter* sp., *Aeromonas* sp., *Bacillus, C. indologenes, C. freundii, E. coli, H. alvei, K. pneumoniae, P. shigelloides, Proteus* sp., *Serratia* sp., *S. putrefaciens, Streptococcus* sp., *Vibrio* sp.) (Austin and Austin, 1989; Inglis *et al.*, 1993; MacMillan, 1985).

In general, the number and types of bacteria isolated from WC could reflect: (1) The physico-chemical conditions of the aquatic environment, e.g., temperature, pH, salinity; (2) Interactions between microorganisms and catfish, e.g., location of bacteria, resident microflora, transient residents; (3) Harvesting, e.g., catching, checking for off-flavor; (4) Handling, e.g., transportation, packaging; (5) The methods employed for isolation, e.g., types of media used, temperature and duration of incubation, the nature of the gaseous environment in the incubator; and (6) The method of identification, e.g., the Vitek^{TM} system.

Table 5. Types of bacteria isolated from whole catfish (WC) and catfish fillets (CF)

Type of bacteria	Isolated from WC	Isolated from CF
Acinetobacter baumannii	-[a]	+[b]
Acinetobacter lwoffii	+	+
Actinobacillus ureae	-	+
Aeromonas hydrophila	+	+
Aeromonas veroni biovar sobria	+	+
Bordetella bronchiseptica	-	+
Cedecea lapagei	-	+
Chromobacterium violaceum	+	-
Chryseobacterium (Flavobacterium) indologenes	+	+
Citrobacter freundii	+	+
Comamonas acidovorans	-	+
Corynebacterium xerosis	-	+
Enterobacter cloacae	-	+
Enterococcus avium (Group D)	-	+
Enterococcus durans (Group D)	-	+
Enterococcus faecalis (Group D)	-	+
Enterococcus faecium (Group D)	-	+
Enterococcus hirae (Group D)	-	+
Escherichia coli	+	+
Hafnia alvei	+	-
Klebsiella pneumoniae	-	+
Listeria species	-	+

(TABLE continued)

Morganella (Proteus) morganii	+	+
Nonfermenting Gram-negative Bacillus (saccharolytic)	-	+
Nonfermenting Gram-negative Bacillus (asaccharolytic)	-	+
Pasteurella haemolytica	+	-
Plesiomonas shigelloides	-	+
Proteus vulgaricus	-	+
Providencia (Proteus) alcalifaciens	+	+
Providencia (Proteus) rettgeri	-	+
Serratia liquefaciens	-	+
Serratia odorifera	+	-
Shewanella putrefaciens	-	+
Staphylococcus aureus	-	+
Staphylococcus auricularis	+	+
Staphylococcus capitis	-	+
Staphylococcus cohnii	-	+
Staphylococcus haemolyticus	-	+
Staphylococcus hominis	-	+
Staphylococcus hyicus	-	+
Staphylococcus lentus	+	-
Staphylococcus saprophyticus	-	+
Staphylococcus sciuri	+	+
Staphylococcus xylosus	+	-
Stenotrophomonas (Xanthomonas) maltophilia	-	+

(TABLE continued)

Streptococcus agalactiae	+	-
Streptococcus anginosus	-	+
Vibrio alginolyticus	+	+
Vibrio cholerae	-	+
Vibrio fluviales	+	+
Vibrio parahemolyticus	+	-

[a](-); the isolate was not present.
[b](+); the isolate was present.

The majority of bacterial isolates (forty three) were isolated and identified from CF. When comparing the number of bacterial isolates of WC and CF, it appears that the higher number of bacteria isolated from CF is not only determined by the natural microflora of WC, but also from other potential sources associated with catfish processing such as workers, processing equipment, surfaces, utensils, packaging materials, sellers, and air (Garbutt, 1997). One needs to take in consideration that the flesh of live fish is bacteriologically sterile; however, fillets are usually contaminated during processing, particularly in the gutting and filleting operations (Garbutt, 1997; Nickelson and Finne, 1992). In addition, CF are an excellent nutrient-rich substrate for growth of psychrotrophic pathogens (Fernandez *et al.*, 1998b).

Selective and nonselective culture media used for isolation. Both selective and nonselective culture media were suitable for isolation of endogenous bacterial flora from WC and CF (Table 6). The most frequently isolates either on selective and nonselective culture media were *Aeromonas* sp. MacMillan (1985) consistently isolated *Aeromonas* sp. from the catfish viscera throughout an entire year of catfish production.

In general, most of bacterial isolates either nonpathogenic or pathogenic were isolated on selective media. For example, CIN (BBL) agar was suitable for the isolation of *Aeromonas* sp., *C. freundii* and *M. morganii* from WC and CF; and *Nonferm. Bacillus* (asaccharolytic) from CF based on mannitol fermentation. This fermentation

results in acid production with a subsequent change of the indicator neutral red to red color (pH 6.8). In addition to mannitol as a carbon source, the medium contains selective compounds such as sodium desoxycholate (toxic chemical); cefsulodin, irgasan and novobiocin (antibiotics); and crystal violet (toxic dye) to inhibit *E. coli, Klebsiella, Proteus, Pseudomonas,* and Gram-positive bacteria (Atlas, 1997; Koneman *et al.*, 1992; Lyon, 1998).

Table 6. Selective and nonselective media used to isolate bacteria from whole catfish (WC) and catfish fillets (CF)

Media[a]	Bacteria
CIN	*A. hydrophila, A. sobria, C. freundii, M. morganii,* and *Nonferm. Bacillus* (asaccharolytic).
MYP	*A. hydrophila, A. sobria, C. violaceum, C. xerosis, V. alginolyticus,* and *V. parahaemolyticus.*
MCPC	*A. sobria, H. alvei, M. morganii, P. alcalifaciens, S. odorifera, S. xylosus,* and *V. cholerae.*
MOX	*A. lwoffii, C. xerosis, Listeria* sp., *M. morganii, P. rettgeri, S. auricularis, S. haemolyticus, S. hominis, S. saprophyticus, S. sciuri,* and *V. alginolyticus.*
PF	*A. ureae, A. hydrophila, A. sobria, C. (Flavobacterium) indologenes, C. acidovorans, C. xerosis,* and *S. aureus.*
SimPlate	*E. coli.*
SA	*A. hydrophila* and *S. auricularis.*
SPS	*A. hydrophila, C. lapagei, E. faecium, P. vulgaricus, S. capitis, S. cohnii,* and *V. cholerae.*
TCBS	*A. sobria, E. avium, E. faecalis, E. hirae, P. shigelloides, P. alcalifaciens, S. lentus, S. sciuri,* and *V. cholerae.*

(TABLE continued)

TSA	*A. lwoffii, A. hydrophila, A. sobria, B. bronchiseptica, E. cloacae, Nonferm. Bacillus* (asaccharolytic), *S. putrefaciens, S. auricularis, S. capitis, S. cohnii,* and *V. fluviales.*
TSC	*A. hydrophila, A. sobria, C. freundii, C. xerosis, E. durans, Nonferm. Bacillus* (saccharolytic), *P. haemolytica, S. liquefaciens, S. aureus, S. auricularis, S. hyicus, S. maltophilia, S. anginosus,* and *V. alginolyticus.*
VRB	*A. hydrophila, A. sobria, B. bronchiseptica, C. freundii, E. coli, K. pneumoniae, S. liquefaciens, V. alginolyticus,* and *V. fluviales.*
XLD	*A. baumannii, A. hydrophila, A. sobria, P. shigelloides, S. agalactiae, V. fluviales,* and *V. parahaemolyticus.*

[a] Incubation in aerobic conditions at 37°C; except MYP (Difco) and CIN (BBL) at 30°C, and MCPC (Difco) at 40°C.

MYP (Difco) agar isolated *Aeromonas* sp. and *V. alginolyticus* from WC and CF. *C. violaceum* and *V. parahaemolyticus* were isolated from WC, as well as *C. xerosis* from CF. MYP uses mannitol fermentation as an indicator for the ability of those isolates to utilize mannitol. Fermentation results in acid production with a change in the phenol red indicator from red to yellow. In addition to mannitol as a carbon source, the medium contains polymyxin B (antibiotic) as a selective agent to inhibit Gram-negative bacilli (except *Pseudomonas*) and Gram-positive cocci, and egg yolk emulsion as a growth factor to increase the number of *Bacillus cereus* (Atlas, 1997; Koneman *et al.*, 1992; Lyon, 1998).

MCPC (Difco) agar was used to isolate *A. sobria, M. morganii* and *P. alcalifaciens* from WC and CF, and *H. alvei, S. odorifera, S. xylosus* and *V. cholerae* from WC. The medium contains cellobiose as a carbohydrate source, polymyxin B and colistin (antibiotics) as selective compounds to inhibit Gram-positive bacteria. Also, a combination of two pH indicators bromthymol blue and cresol red, and sodium chloride (2%) as a growth factor for halophiles (Atlas, 1997; Koneman *et al.*, 1992; Lyon, 1998).

On MOX (Oxoid) agar, *A. lwoffii, M. morganii, S. auricularis, S. sciuri* and *V. alginolyticus* were isolated from WC and CF, and *C. xerosis, Listeria* sp., *P. rettgeri, S. haemolyticus, S. hominis* and *S. saprophyticus* from CF. The medium contains antibiotics such as cycloheximide, colistin sulfate, fosfomycin, acriflavine and cefotetan, and other inhibitory compounds such as sodium chloride and lithium chloride as selective agents. In addition, the medium contains a double indicator system. The first indicator is esculin and ferric ammonium citrate. *Listeria* sp. hydrolyzes esculin to esculetin which reacts with ferric citrate to form a black or dark complex. The second indicator system is mannitol and phenol red. Typical contaminants such as *Staphylococcus* are mannitol positive, which results in acid production and a change in the agar color from red to yellow (Atlas, 1997; Koneman *et al.*, 1992; Lyon, 1998).

On PF (Acumedia) agar, *Aeromonas* sp. and *C. (Flavobacterium) indologenes* were isolated from WC and CF, and *A. ureae, C. acidovorans, C. xerosis* and *S. aureus* from CF on the basis of fluorescein production (Atlas, 1997).

Total coliform & *E. coli* Simplate (IDEXX) was used to detect and quantify the total coliform and *E. coli* from WC and CF. Simplate is based on a patented defined substrate technology which equates the presence of total coliform and *E. coli* to the presence of β-galactosidase and β-glucuronidase, respectively.

On SA (Difco) agar, *A. hydrophila* and *S. auricularis* were isolated from WC and CF on the basis of α-amylase production. The medium contains starch which is hydrolyzed by α-amylase. A clear zone around the colonies indicates that α-amylase has been produced by the bacteria. In addition to starch, ampillicin (antibiotic) is used as a selective compound (Atlas, 1997; Lyon, 1998).

SPS (Difco) agar was used for anaerobic isolation of *A. hydrophila* from WF and CF, and *C. lapagei, E. faecium, P. vulgaricus, S. capitis, S. cohnii* and *V. cholerae* from CF. The medium contains antibiotics (e.g., polymyxin B and sulfadiazine) as selective

agents, and ferric citrate and sodium sulfite are used as the differential system (Atlas, 1997; Lyon, 1998).

TCBS (Difco) agar was used to isolate *A. sobria, P. alcalifaciens* and *S. sciuri* from WC and CF, *E. avium, E. faecalis, E. hirae, P. shigelloides* and *V. cholerae* from CF, and *S. lentus* from WC. Even though *A. sobria, P. shigelloides* and *V. cholerae* are non-halophilic, they were able to grow on this medium. TCBS (Difco) contains sodium chloride (2%) to enhance the growth of *Vibrio* sp. and inhibit non halophiles. Oxgall is used for the selection of bile-tolerant bacteria. PH indicators such as bromthymol blue and thymol blue, and hydrogen sulfide indicators such as sodium citrate and sodium thiosulfate are used as differential agents (Lyon, 1998).

TSA (Difco) agar was used as a nonselective agar to isolate *A. lwoffii, A. hydrophila, A. sobria, S. auricularis* and *V. fluviales* from WC and CF, and *B. bronchiseptica, E. cloacae, Nonferm.. Bacillus* (asaccharolytic), *S. putrefaciens, S. capitis* and *S. cohnii* from CF. *A. hydrophila, A. sobria, E. cloacae,* and *Nonferm. Bacillus* (asaccharolytic) were also isolated on TSA under anaerobic conditions. A shortcoming in the identification of bacterial colonies using TSA was the similar appearance of bacteria on the plate; however, it is a good media for determining both aerobic and anaerobic plate counts.

TSC (Oxoid) agar was used for the isolation of anaerobes *A. hydrophila, A. sobria, C. freundii, S. auricularis* and *V. alginolyticus* from WC and CF, *C. xerosis, E. durans, Nonferm. Bacillus* (saccharolytic), *S. liquefaciens, S. aureus, S. hyicus, S. maltophilia* and *S. anginosus* from CF, and *P. haemolytica* from WC. The medium contains a mixture of growth factors (e.g., yeast extract) to increase the number of *C. perfringens*, antibiotics (e.g., cycloserine, polymyxin B) as selective agents, and ferric ammonium citrate and sodium metabisulfide used as the differential system (Atlas, 1997; Lyon, 1998).

On VRB (Difco) agar, *A. hydrophila, A. sobria, C. freundii, E. coli, V. alginolyticus* and *V. fluviales* were isolated from WC and CF, and *B. bronchiseptica, K. pneumoniae* and *S. liquefaciens* from CF. The medium contains lactose to indicate the presence of lactose fermenting enteric organisms. Neutral red is used as a pH indicator to detect acid production. Bile salts and crystal violet (toxic chemicals) are inhibitory agents that inhibit the growth of Gram-positive organisms (Atlas, 1997; Lyon, 1998).

A. baumannii and *P. shigelloides* from CF, *A. hydrophila, A. sobria* and *V. fluviales* from WC and CF, and *S. agalactiae* and *V. parahaemolyticus* from WC were isolated on XLD (Difco) agar. Differentiation of organisms is accomplished by 3 reactions: xylose fermentation, lysine decarboxylation and hydrogen sulfide production. The medium contains lactose, sucrose, xylose and lysine. Sodium desoxycholate is used to inhibit Gram-positive bacteria. Phenol red is used as a pH indicator, and ferric ammonium citrate and sodium thiosulfate as hydrogen sulfide differential indicators (Atlas, 1997; Lyon, 1998).

Colony characteristics such as form, elevation, margin, surface, density, color and size of bacterial isolates from both WC and CF on all selective and nonselective media are shown in Table 7.

Table 7. Colony characteristics of bacterial isolates from whole catfish (WC) and catfish fillets (CF) on selective and nonselective media

Bacterial isolate	Colony characteristics
A. baumannii	Round, raised, opaque, white colonies of 4 mm in diam on XLD (Difco).
A. lwoffii	Round, raised, translucent, brown nucleus, white colonies of 4 mm in diam on TSA (Difco) in aerobic and anaerobic conditions. Round, raised, brown colonies of 3 mm in diam on MOX (Oxoid).

(TABLE continued)

A. ureae	Round, raised, white colonies of 3 mm in diam on PF (Acumedia).
A. hydrophila	Round, raised, opaque, red colonies of 3 mm in diam on CIN (BBL). Round, raised, ondulate, yellow colonies of 2 mm in diam on MYP (Difco). Round, raised, ondulate, white colonies of 6 mm in diam on PF (Acumedia). Round, raised, white colonies of 2 mm in diam on SA (Difco). Irregular, flat, black colonies of 3 mm in diam on SPS (Difco) anaerobically. Round, raised, translucent, creamy colonies of 2 mm in diam on TSA (Difco). Round, raised, opaque, brown colonies of 2 mm in diam on TSC (Oxoid) anaerobically. Round, raised, opaque, red colonies of 2-4 mm in diam on VRB (Difco). Round, raised, opaque, shiny, yellow colonies of 2-4 mm in diam with a yellow halo on XLD (Difco).
A. sobria	Round, raised, entire, opaque, brown colonies of 3 mm in diam on CIN (BBL). Punctiform, pulvinate, yellow colonies of 1 mm in diam on MYP (Difco). Round, raised, dark nucleus, green colonies of 3 mm in diam on MCPC (Difco). Round, opaque, white colonies of 2 mm in diam on PF (Acumedia). Round, raised, opaque, creamy colonies of 4 mm in diam on TSA (Difco) in aerobic and anaerobic conditions. Irregular, raised, opaque, creamy colonies of 2 mm in diam surrounded by a zone of precipitate on TSC (Oxoid) anaerobically. Round, raised, opaque, yellow colonies of 3 mm in diam on TCBS (Difco). Round, raised, opaque, red colonies of 2 mm in diam on VRB (Difco).
B. bronchiseptica	Round, raised, opaque, brown colonies of 3 mm in diam on TSA (Difco). Round, raised, opaque, gray colonies of 1 mm in diam on VRB (Difco).
C. lapagei	Round, raised, transparent, creamy colonies of 4 mm in diam on SPS (Difco) anaerobically.
C. violaceum	Irregular, brown colonies of 2 mm in diam on MYP (Difco).
C. indologenes	Round, raised, opaque, white colonies of 2 mm in diam on PF (Acumedia). Round, raised, opaque, yellow colonies of 2 mm in diam on TSC (Oxoid) anaerobically.
C. freundii	Round, raised, opaque, pink nucleus, brick red colonies of 3 mm in diam on CIN (BBL). Round, convex, entire, black nucleus,

(TABLE continued)

	brown colonies of 2 mm in diameter on TSC (Oxoid) anaerobically. Round, raised, red colonies of 4 mm in diam with nucleus on VRB (Difco).
C. acidovorans	Round, raised, opaque, white colonies of 3 mm in diam on PF (Acumedia).
C. xerosis	Round, raised, entire, opaque, yellow colonies of 1 mm in diam on MOX (Oxoid). Round, raised, white colonies of 2 mm in diam with nucleus on PF (Acumedia). Round, flat, entire, opaque, yellow colonies of 2 mm in diam on MYP (Difco). Round, raised, entire, opaque, yellow colonies of 2 mm in diam on TSC (Oxoid) anaerobically.
E. cloacae	Round, raised, white colonies of 2 mm in diam on TSA (Difco) anaerobically. Round, raised, opaque, yellow colonies of 4 mm in diam on XLD (Difco).
E. avium	Round, raised, opaque, green colonies of 1 mm in diam on TCBC (Difco).
E. durans	Round, raised, white colonies of 2 mm in diam on TSC (Oxoid) anaerobically.
E. faecalis	Round, raised, opaque, yellow colonies of 2 mm in diam on TCBC (Difco).
E. faecium	Round, flat, opaque, creamy colonies of 3 mm in diam on SPS (Difco) anaerobically.
E. hirae	Round, raised, entire, opaque, yellow colonies of 2 mm in diam on TCBC (Difco).
E. coli	Round, raised, red colonies of 2 mm in diam with nucleus on VRB (Difco) .
H. alvei	Round, white colonies of 2 mm in diam on MCPC (Difco).
K. pneumoniae	Round, raised, pulvinate, red colonies of 4 mm in diam on VRB (Difco).
Listeria sp.	Punctiform, pulvinate, opaque, brown colonies of 1 mm in diam on MOX (Oxoid).

(TABLE continued)

M. morganii	Round, raised, opaque, brick read colonies of 2 mm in diam on CIN (BBL). Round, raised, ondulate, opaque, green colonies of 1 mm in diam on MOX (Oxoid). Round, raised, opaque, blue colonies of 2 mm in diam on MCPC (Difco).
Nonferm. Bacillus (saccharolytic)	Round, raised, opaque, brown colonies of 2 mm in diam on TSC (Oxoid) anaerobically.
Nonferm. Bacillus (asaccharolytic)	Round, raised, opaque, read colonies of 2 mm in diam on CIN (BBL). Round, raised, yellow colonies of 3 mm in diam on TSA (Difco).
P. haemolytica	Round, black colonies of 1 mm in diam on TSC (Oxoid) anaerobically.
P. shigelloides	Round, raised, dark green colonies of 2 mm in diam on TCBC (Difco). Round, raised, opaque, red colonies of 2 mm in diam on XLD (Difco).
P. vulgaricus	Round, raised, opaque, white colonies of 1 mm in diam on SPS (Difco) anaerobically.
P. alcalifaciens	Round, raised, entire, green nucleus, light green colonies of 2 mm in diam on MCPC (Difco). Round, raised, transparent, creamy colonies of 2 mm in diam on SPS (Difco) anaerobically. Round, raised, entire, opaque, green colonies of 2 mm in diam on TCBS (Difco).
P. rettgeri	Punctiform, pulvinate, entire, opaque, brown colonies of 1 mm in diam on MOX (Oxoid).
S. liquefaciens	Round, raised, opaque, white colonies of 2 mm in diam on TSC (Oxoid) anaerobically. Round, raised, opaque, purple colonies of 2 mm in diam on VRB (Difco).
S. odorifera	Round, white colonies of 2 mm in diameter on MCPC (Difco).
S. putrefaciens	Round, raised, opaque, creamy colonies of 3 mm in diam on MYP (Difco). Round, raised, ondulate, brown colonies of 3 mm in diam on TSA (Difco).
S. aureus	Round, raised, ondulate, opaque, yellow colonies of 1 mm in diam on PF (Acumedia). Round, raised, opaque, creamy colonies of 1 mm in diam on TSC (Oxoid) anaerobically.

(TABLE continued)

S. auricularis	Round, raised, ondulate, opaque, yellow colonies of 1 mm in diam on MOX (Oxoid) agar. Round, raised, yellow colonies of 2 mm in diam on SA (Difco). Round, raised, brown colonies of 2 mm in diam on TSA (Difco). Round, raised, opaque, white colonies of 1 mm in diam on TSC (Oxoid) anaerobically.
S. capitis	Round, raised, entire, creamy colonies of 2 mm in diam on SPS (Difco) anaerobically. Round, flat, entire, opaque, yellow colonies of 3 mm in diam on TSA (Difco).
S. cohnii	Round, raised, entire, creamy colonies of 2 mm in diam on SPS (Difco) anaerobically. Round, flat, entire, opaque, white colonies of 3 mm in diam on TSA (Difco).
S. haemolyticus	Round, raised, opaque, yellow colonies of 1 mm in diam on MOX (Oxoid).
S. hominis	Round, raised, opaque, white colonies of 1 mm in diam on MYP (Difco).
S. hyicus	Round, raised, ondulate, opaque, yellow colonies of 2 mm in diam on TSC (Oxoid) anaerobically.
S. lentus	Round, raised, yellow colonies of 2 mm in diam on TCBS (Difco).
S. saprophyticus	Round, raised, opaque, yellow colonies of 1 mm in diam on MOX (Oxoid).
S. sciuri	Punctiform, pulvinate, opaque, brown colonies of 1 mm in diam on MOX (Oxoid). Punctiform, raised, opaque, brown colonies of 2 mm in diam on TCBS (Difco). Punctiform, raised, opaque, white colonies of 2 mm in diam on TSA (Difco) anaerobically. Punctiform, raised, opaque, white colonies of 2 mm in diam on SPS (Difco) anaerobically.
S. xylosus	Irregular, blue colonies of 2 mm in diam on MCPM (Difco).
S. maltophilia	Round, raised, opaque, white colonies of 1 mm in diam on TSC (Oxoid) anaerobically.
S. agalactiae	Round, raised, red nucleus, slightly brown colonies of 3 mm in diam on XLD (Difco).

(TABLE continued)

S. anginosus	Punctiform, raised, opaque, yellow colonies of 1 mm in diam on TSC (Oxoid) anaerobically.
V. alginolyticus	Round, raised, opaque, yellow colonies of 1 mm in diam on MYP (Difco). Round, raised, creamy colonies of 2 mm in diam on MOX (Oxoid). Round, raised, opaque, yellow colonies of 3 mm in diam on TSA (Difco). Round, raised, opaque, brown colonies of 2 mm in diam on TSC (Oxoid) anaerobically. Round, raised, opaque, red colonies of 3 mm in diam on VRB (Difco).
V. cholerae	Round, raised, entire, opaque, green colonies of 1 mm in diam on MCPC (Difco). Round, flat, ondulate, translucent, light brown colonies of 3 mm in diam on SPS (Difco) anaerobically. Round, convex, entire, opaque, yellow colonies of 2 mm in diam on TCBS (Difco).
V. fluviales	Round, raised, opaque, brown colonies of 2-4 mm in diam on TSA (Difco). Round, flat, ondulate, opaque, yellow colonies of 2 mm in diam on XLD (Difco). Round, red nucleus, white colonies of 4 mm on VRB.
V. parahemolyticus	Round, raised, brown colonies of 1 mm on MYP (Difco) anaerobically. Round, raised, white colonies of 2 mm in diam on XLD (Difco).

Gram-positive and Gram-negative bacteria. Twenty bacterial isolates from WC and CF, approximately 40% of all isolates, were Gram-positive bacteria. Thirty-one Gram-negative bacteria accounted for the remaining 60% of all isolates. Table 8 shows the phenotypic characteristics of individual bacterial isolates, using the Gram-stain reaction, oxidase test, catalase test, and coagulase reaction.

The Gram-stain reaction differentiated Gram-positive from Gram-negative bacteria from WC and CF on the basis of differences in cell wall composition and thus differences in cellular permeability to a solvent such as ethanol. The higher content of cell-wall peptidoglycan, lower lipid content, and absence of lipopolysaccharide in the Gram-positive bacteria cell wall are responsible for their Gram stain characteristics. Unlike the Gram-negative bacteria, Gram-positive bacterial cells retain the crystal

violet-iodine complex because their carbohydrate-rich cell walls are not disorganized by the alcohol decolorizing agent used in the Gram-stain procedure. The integrity of the lipid- and lipopolysaccharide-rich cell wall of Gram-negative bacteria is easily disrupted by ethanol and the crystal violet-iodine complex is leached from the bacterial cell (Koneman *et al.*, 1992; Lyon, 1998; Poelma and Bryce, 1992).

Table 8. Phenotypic characteristics of bacterial isolates using the Gram stain reaction, oxidase test, catalase test and coagulase from whole catfish (WC) and catfish fillets (CF)

Bacterial isolate	Phenotypic test	
Gram-negative	**Gram stain**	**Oxidase**
A. baumannii	Pink, rods, short chains, monolayer.	-[a]
A. lwoffii	Pink, rods, long chains, monolayer.	-
A. ureae	Pink, rods, short chains, multilayer.	+[b]
A. hydrophila	Pink, rods, singly, in pairs or short chains.	+
A. sobria	Pink, rods, singly, in pairs or short chains.	+
B. bronchiseptica	Pink, rods, short chains, monolayer.	+
C. lapagei	Pink, rods, short chains, monolayer.	-
C. violaceum	Pink, rods, short chains, monolayer.	+
C. indologenes	Pink, rods, short chains, monolayer.	+
C. freundii	Pink, rods, short chains, monolayer.	-
C. acidovorans	Pink, rods, short chains, monolayer.	+
E. cloacae	Pink, rods, short chains, monolayer.	-
E. coli	Pink, rods, singly or in pairs.	-
H. alvei	Pink, rods, short chains.	-
K. pneumoniae	Pink, rods, singly, in pairs or short chains.	-

(TABLE continued)

M. morganii	Pink, rods, in pairs or short chains.	-
Nonferm. Bacillus (saccharolytic)	Pink, rods, short chains.	+
Nonferm. Bacillus (asaccharolytic)	Pink, rods, long chains, monolayer.	+
P. haemolytica	Pink, rods, short and long chains.	-
P. shigelloides	Pink, rods, in pairs or short chains.	+
P. vulgaricus	Pink, rods, long chains, monolayer.	-
P. alcalifaciens	Pink, rods, short and long chains.	-
P. rettgeri	Pink, rods, short chains.	-
S. liquefaciens	Pink, rods, short chains, monolayer.	-
S. odorifera	Pink, rods, singly or in pairs.	-
S. putrefaciens	Pink, rods, long chains, monolayer.	+
S. maltophilia	Pink, rods, long chains, monolayer.	-
V. alginolyticus	Pink, rods, in pairs or short chains.	+
V. cholerae	Pink, rods, short chains, monolayer.	+
V. fluviales	Pink, rods, short chains, monolayer.	+
V. parahemolyticus	Pink, rods, singly.	+

Gram-positive	**Gram stain**	**Catalase**	**Coagulase**
C. xerosis	Purple, coccus, long chains, multilayer.	+[c]	-[e]
E. avium	Purple, coccus, short and long chains.	-[d]	-
E. durans	Purple, coccus, long chains, multilayer.	-	-
E. faecalis	Purple, coccus, long chains, multilayer.	-	-
E. faecium	Purple, coccus, long chains, multilayer.	-	-
E. hirae	Purple, coccus, long chains, multilayer.	-	-
Listeria sp.	Purple, rods, short chains, monolayer.	+	-

(TABLE continued)

S. aureus	Purple, coccus, in pairs and clusters.	+	+[f]
S. auricularis	Purple, coccus, short and long chains.	+	-
S. capitis	Purple, coccus, long chains, clusters.	+	-
S. cohnii	Purple, coccus, long chains, clusters.	+	-
S. haemolyticus	Purple, coccus, short chains, multilayer.	+	-
S. hominis	Purple, coccus, short chains, multilayer.	+	-
S. hyicus	Purple, coccus, clusters.	+	+
S. lentus	Purple, coccus, short chains.	+	-
S. saprophyticus	Purple, coccus, singly, in pairs, tetrads.	+	-
S. sciuri	Purple, coccus, short and long chains.	+	-
S. xylosus	Purple, coccus, singly, in pairs.	+	-
S. agalactiae	Purple, coccus, in pairs or short chains.	-	-
S. anginosus	Purple, coccus, short chains, bilayer.	-	-

[a](-); oxidase test was negative.
[b](+); oxidase test was positive.
[c](+); catalase test was positive.
[d](-); catalase test was negative.
[e](-); coagulase was negative.
[f](+); coagulase was positive.

It has been stated that Gram-negative bacteria are the cause of the majority of bacterial diseases associated with fish (Austin and Austin, 1989; Frerichs, 1993). Austin and Austin (1989) reported that only few Gram-positive bacteria are organisms of disease importance in fish. In addition, growth of Gram-negative bacteria in refrigerated catfish fillets is the primary factor that determines spoilage of fresh fillets (Kim *et al.*, 1995a).

Some Gram-positive bacteria (e.g., *S. aureus*) are associated with human infections diseases. These pathogenic bacteria may originate from the gut of WC or may be due to cross contamination from workers' hands and skin. The relatively high

tolerance of most Gram-positive bacteria against limiting factors such as a reduced water activity, refrigeration temperatures and reduced pH, allows a higher survival rate and longer persistance as compared to most Gram-negative bacteria (Ray, 1996). Gram-negative bacteria are normally more sensitive to lower pH than Gram-positive bacteria (Kim *et al.*, 1995a).

Classification of bacteria. Bacteria isolated from WC and CF were classified into groups and families based on the Shorter Bergey's Manual of Determinative Bacteriology (Holt, 1977; Table 9). The phenotypic characteristics of bacterial isolates, e.g., the Gram-stain reaction, oxidase test, catalase test, coagulase, were considered to assign bacteria to a family or group. For example, *Enterobacteriaceae*, *Pseudomonadaceae*, and *Vibrionaceae* families are differentiated by the oxidase test. Organisms belonging to the *Pseudomonadaceae* and *Vibrionaceae* families are oxidase-positive, while organisms of the *Enterobacteriaceae* family are oxidase-negative. Frerichs (1993) reported that the majority of fish pathogens are Gram-negative rods belonging to one of these three families. These bacteria are causative agents responsible for acute septicemias with few symptoms and high mortalities, chronic conditions and low mortalities, or asymptomatic latent infections. In addition to those families, *Aeromonas* sp. belonging to the *Vibrionaceae* family has been proposed to be elevated to the family status of *Aeromonadaceae* (Austin and Austin, 1989).

On the other hand, *Micrococcaceae* and *Streptococcaceae* families are differentiated by the catalase test. Microorganisms belonging to the *Micrococcaceae* family are catalase-positive, while microorganisms belonging to the *Streptococcaceae* family are catalase-negative. Coagulase-positive *S. aureus* is separated from coagulase-negative *Staphylococcus* sp. by the coagulase test.

Table 9. Classification of bacteria into groups and families from whole catfish (WC) and catfish fillets (CF)

Group	Family	Bacteria
Gram-negative aerobic rods	*Pseudomonadaceae*	*C. acidovorans, S. putrefaciens,* and *S. maltophilia.*
	Other Genera	*B. bronchiseptica.*
Gram-negative facultatively anaerobic rods	*Enterobacteriaceae*	*C. freundii, E. cloacae, E. coli, H. alvei, K. pneumoniae, M. morganii, P. vulgaricus, P. alcalifaciens, P. rettgeri, S. liquefaciens,* and *S. odorifera.*
	Vibrionaceae	*A. hydrophila, A. sobria, P. shigelloides, V. alginolyticus, V. cholerae, V. fluviales,* and *V. parahemolyticus.*
	Other Genera	*A. ureae, C. lapagei, C. violaceum, C. indologenes, Nonferm. Bacillus* (saccharolytic and asaccharolytic), and *P. haemolytica.*
Gram-negative cocci	*Neisseriaceae*	*A. baumannii* and *A. lwoffii.*
Gram-positive facultatively anaerobic cocci	*Micrococcaceae*	*S. aureus, S. auricularis, S. capitis, S. cohnii, S. haemolyticus, S. hominis, S. hyicus, S. lentus, S. saprophyticus, S. sciuri,* and *S. xylosus.*
	Streptococcaceae	*E. avium, E. durans, E. faecalis, E. faecium, E. hirae, S. agalactiae,* and *S. anginosus.*
Gram-positive rods	Coryneform bacteria	*C. xerosis.*
	Other Genera	*Listeria* sp.

Significance of bacterial isolates. From fifty-one bacterial species isolated from WC and CF, only few bacteria such as, *Acinetobacter* sp. (Austin and Austin, 1993) and *Aeromonas* sp. (Austin and Austin, 1993; MacMillan, 1985; Stickney, 1991;

Wedemeyer, 1997) have been associated with diseases in channel catfish. Important catfish pathogens, e.g., *Edwardsiella ictaluri, E. tarda,* were not isolated from WC nor CF. However, other bacteria which are considered pathogens of freshwater and/or marine fish species were isolated from our samples. Table 10 shows bacterial fish pathogens from WC and CF and associated diseases. Detailed information about bacterial diseases of fish can be found in Austin and Austin (1993), Austin and Austin (1989), and Inglis *et al.* (1993).

Table 10. Bacterial fish pathogens from whole catfish (WC) and catfish fillets (CF) and associated diseases

Bacterial pathogen	Disease
Acinetobacter sp.	Acinetobacter disease
Aeromonas sp.	Aeromonas septicemia
Citrobacter freundii	Secondary infection
Hafnia alvei	Haemorrhagic septicemia
Plesiomonas shigelloides	Septicemia
Providencia rettgeri	Septicemia
Serratia liquefaciens	Serratia septicemia
Shewanella putrefaciens	Septicemia
Staphylococcus aureus	Eye disease
Streptococcus agalactiae	Septicemia
V. alginolyticus	Vibriosis
V. cholerae	Vibriosis
V. parahaemolyticus	Vibriosis

On the other hand, it has been stated that endogenous microflora of fish may be transferred to human beings and produce several diseases. Human pathogens isolated from WC and CF, and their infections are shown in Table 11 (Austin and Austin, 1989; Inglis *et al.*, 1993).

Table 11. Bacteria of significance as human pathogens isolated from whole catfish (WC) and catfish fillets (CF)

Human pathogen	Disease
Acinetobacter sp.	Hospital-acquired (nosocomial) infections
A. ureae	Bronchiectasis and chronic bronchitis
Aeromonas sp.	Gastroenteritis; blood infection (septicemia)
B. bronchiseptica	Whooping cough
C. violaceum	Septicemia
C. indologenes	Septicemia; meningitis
C. freundii	Gastroenteritis; histamine intoxication
E. cloacae	Histamine intoxication
E. coli	Gastroenteritis
H. alvei	Histamine intoxication
K. pneumoniae	Gastroenteritis; histamine intoxication
Listeria sp.	Uterine infections; abortion; still borns
M. morganii	Histamine intoxication; urinary infection
P. shigelloides	Gastroenteritis
Proteus sp.	Histamine intoxication; urinary-tract infections
S. aureus	Intoxication and gastrointestinal symptoms
Vibrio sp.	Gastroenteritis; histamine intoxication

According to Bean and Griffin (1990) some human pathogens (e.g., *A. hydrophila, E. coli, P. shigelloides, Proteus* sp., *S. aureus, V. fluvialis, V. cholerae)* have been causative agents of foodborne outbreaks in the U.S. between 1973 and 1987. They also reported that fish was a major food vehicle for foodborne illness. Improper storage or holding temperature was the factor most often reported for outbreaks caused by fish. In addition, some food pathogens can grow at refrigeration temperatures such as *A. hydrophila, E. coli, Listeria* sp., *P. shigelloides, Proteus* sp., *S. aureus,* and *V. parahaemolyticus* (Garbutt, 1997; Ray, 1996).

Other bacteria isolated from WC and CF which do not represent a health hazard but they are associated with the spoilage of catfish products include: *Acinetobacter* sp., *Aeromonas* sp., *C. indologenes, C. freundii, H. alvei, Proteus* sp., *S. putrefaciens,* and *Vibrio* sp. (Austin and Austin, 1989; Davies, 1997; Nickelson and Finne, 1992). Other microorganisms, predominantly Gram-positive bacteria such as *Bacillus, Enterococcus* and *Staphylococcus* are artificially introduced to catfish and reflect the microflora of diets used to feed catfish.

Enumeration. Aerobic and anaerobic counts (CFU/g) of WC and CF on selective and nonselective media are shown in Table 12. APC counts from WC and CF showed that catfish samples were highly contaminated. The high APC value indicated that both WC and CF are highly from several potential sources, including aquatic environment, holding tanks, and processing.

The coliform counts and the presence of *E. coli* may indicate contamination from fecal water, or from the evisceration process. The fact that the WC are contaminated with *E. coli* indicates that *E. coli* was introduced in the pond via fecal contamination or introduced in the holding tanks at the plant. Andrews *et al.* (1977) surveyed 41 processors to determine the bacteriological quality of fresh catfish. They observed that the APC ranged from 6.9×10^3 to 1.9×10^8 CFU/g, total coliforms from <3 to 9.3×10^3 CFU/g, and fecal coliforms from <3 to 4.6×10^2 CFU/g. It is important

to consider that the high number of microorganisms in WC and CF along with the presence of psychrotrophic bacteria equate to the possibility of a high rate of spoilage, and thus a shorter shelf-life of chilled catfish products.

The presence of *E. coli* in WC and CF indicates there may be a potential health hazard to consumers if not cooked thoroughly. WC and CF bacterial counts may be considered acceptable at these microbial loads since the maximum limit recommended by the International Commission on Microbiological Specifications for Foods (ICMSF, 1986) for fresh fish is 10^7 CFU/g.

Table 12. Aerobic and anaerobic counts (CFU/g) of whole catfish (WC) and catfish fillets (CF) on selective and nonselective media

Microbial test	WC (CFU/g)	CF (CFU/g)
APC (aerobic)	8.4×10^6	2.6×10^7
APC (anaerobic)	1.3×10^6	1.3×10^7
Coliforms	7.5×10^5	7.4×10^3
E. coli	7.4×10^3	3.2×10^3

Evaluation of the Grovac Processing Method

Experiment 1. The individual and combination effects of 0.4% AA plus 0.4% NaCl and vacuum (28 in. Hg) on the reduction of microbial load in CF are shown in Table 13. The data revealed that various combinations of the processing parameters were effective in the reducing of microbial load in CF using the Grovac process (Table 13). The highest log reduction by 2 log CFU/g units was achieved by combining 0.4% AA plus 0.4% NaCl and vacuum (28 in. Hg) (Table 13, Treatment D). The bacterial reduction was apparently due to the acidic conditions (pH 3.4) of the process solution under vacuum. Lyon (1998) and Ray (1996) reported that the bactericidal effect is due to disruption of both the cell membrane integrity, and the proton pump.

Table 13. Log CFU/g reduction of the aerobic plate counts (APC) for catfish fillets (CF) treated individually or combination with various process parameters

Treatment & Process parameters[a]	Log initial	Log final	Log reduction[b]
A: Water	8.35 [c] (0.18)	7.13 [cd] (0.31)	1.22 [d] (0.20)
B: Water + 0.4% AA + 0.4% NaCl	8.43 [c] (0.10)	7.20 [cd] (0.28)	1.23 [d] (0.23)
C: Water + vacuum (28 in. Hg)	8.33 [c] (0.15)	7.53 [c] (0.19)	1.28 [d] (0.17)
D: Water + 0.4% AA + 0.4% NaCl + vacuum (28 in. Hg)	8.45 [c] (0.06)	6.48 [d] (0.42)	1.97 [c] (0.37)

[a] Variable process parameters include water, ascorbic acid (AA; 0.4% w/v), and sodium chloride (NaCl; 0.4% w/v), and vacuum (28 in. Hg). Variables that were held constant were tumbling time (8 min) and drum rotation speed (8 rpm).
[b] Mean log values measured in CFU/g. Log reduction = log initial (control) – log final (treated). Numbers in parentheses refer to standard deviation of six measurements.
[c,d] Mean values in a column not followed by the same letter are significantly different ($p<0.05$).

The paired t-test showed that the mean log reduction (mean log initial of the control group minus mean log final of treated group) on CF was significantly greater ($p<0.05$) than zero for all four treatments. The ANOVA showed that the mean log reduction in CF from treatment D (Table 13) was significantly higher ($p<0.05$) than the log reduction means (range from 1.2 to 1.3 CFU/g) of CF treated with 0.4% AA plus 0.4% NaCl, or vacuum (28 in. Hg) alone. However, there was no difference ($p>0.05$) between log reduction means of CF among treatments A, B, and C when compared to that on CF treated with water. These collective data indicate that 0.4% AA plus 0.4% NaCl, or vacuum (28 in. Hg) alone does not effectively reduced log CFU of CF compared to the log CFU of treatment with only water. Therefore, the bacterial

reduction is most likely due to physical removal of bacteria by the water or processing solution. The initial pH (before tumbling of CF) for treatment A, B, C and D was 9.1, 3.4, 9.1 and 3.4, respectively.

It should be noted that during tumbling, the fillets were partially washed with either water or the processing solution while the tumbler was rotated at 8 rpm for 8 min. Thus the increased log reduction of treatment D (Table 13) by an additional 0.7-0.8 log CFU/g with respect to the other treatments may be related to the synergistic effect of tumbling, AA, NaCl and vacuum. This means that the microbial quality of CF was improved by the tumbling effect as well as the lethal and sublethal effect of the processing solution and vacuum on the endogenous microflora of CF.

In conclusion, experiment 1 showed that the overall CF microbial quality could be improved by combining 0.4% AA plus 0.4% NaCl and vacuum (28 in Hg). Since the APC on CF from treatment D was below log 7.0 CFU/g (when spoilage of catfish occurred), the final microbial quality of the product was considered acceptable.

Experiment 2. The effectiveness of 3 levels of AA (0.4, 0.8 and 1.2%) and 3 levels of NaCl (0.2, 0.4 and 0.6%) on the reduction of microbial loads on CF using the Grovac process are shown in Table 14. The ANOVA showed that levels of AA and NaCl were effective in lowering ($p = 0.02$) the initial APC counts in CF by a mean range of 1.2 to 2.3 log reduction (CFU/g). Treatment I with 1.2% AA and 0.6% NaCl achieved the highest mean log reduction of 2.3 log CFU/g compared to those from other treatments. However, mean log reduction of treatment I (Table 14) was not significantly different ($p>0.05$) from other treatments, except treatments A and C. Comparisons among the average log reductions at different levels of AA and NaCl are shown in Table 15. The ANOVA showed that there was a significant AA effect ($p = 0.01$), but not a significant NaCl effect ($p = 0.22$) with respect to log reduction on CF. The highest mean log reduction on CF was achieved by treatments using 1.2% AA; however, it was not significantly different ($p>0.05$) from the treatment using 0.8% AA.

Table 14. Log initial, log final and the average log reduction, and APC reduction of catfish fillets (CF) treated with different levels of ascorbic acid (AA) and sodium chloride (NaCl)

Treatment	Log initial	Log final	Log reduction[a]	APC reduction[b]
A: 0.4% AA + 0.2% NaCl	6.78 ^{fe} (0.44)	5.60 ^{cd} (0.30)	1.18 ^{d} (0.65)	93.4
B: 0.4% AA + 0.4% NaCl	7.58 ^{cd} (0.51)	5.95 ^{c} (0.29)	1.63 ^{cd} (0.58)	97.7
C: 0.4% AA + 0.6% NaCl	6.80 ^{fe} (0.32)	5.52 ^{cd} (0.40)	1.28 ^{d} (0.52)	94.8
D: 0.8% AA + 0.2% NaCl	7.73 ^{c} (0.50)	5.87 ^{c} (0.89)	1.87 ^{cd} (0.57)	98.6
E: 0.8% AA + 0.4% NaCl	6.72 ^{fe} (0.45)	5.22 ^{cd} (0.47)	1.50 ^{cd} (0.42)	96.8
F: 0.8% AA + 0.6% NaCl	7.25 ^{cde} (0.26)	5.28 ^{cd} (0.74)	1.97 ^{cd} (0.70)	98.9
G: 1.2% AA + 0.2% NaCl	6.45 ^{f} (0.34)	4.63 ^{e} (0.44)	1.82 ^{cd} (0.58)	98.5
H: 1.2% AA + 0.4% NaCl	6.92 ^{def} (0.45)	5.42 ^{cd} (0.52)	1.50 ^{cd} (0.37)	96.8
I: 1.2% AA + 0.6% NaCl	7.28 ^{cde} (0.28)	4.98 ^{cd} (0.52)	2.30 ^{c} (0.35)	99.5

[a] Mean log values measured in CFU/g. Log reduction = log initial (control) – log final (treated). Numbers in parentheses refer to standard deviation of six measurements.
[b] APC reduction (%) = (initial CFU/g – final CFU/g) (100) / initial CFU/g.
[c,d,e,f] Mean values in a column not followed by the same letter are significantly different ($p<0.05$).

Table 15. Log reduction means on catfish fillets (CF) treated with different levels of ascorbic acid (AA) and sodium chloride (NaCl)

Level of AA (%)	Log reduction[a]
0.4	1.37 [c] (0.59)
0.8	1.78 [bc] (0.58)
1.2	1.87 [b] (0.54)
Level of NaCl (%)	**Log reduction[a]**
0.2	1.62 [b] (0.67)
0.4	1.54 [b] (0.44)
0.6	1.85 [b] (0.67)

[a] Mean log values measured in CFU/g. Log reduction = log initial (control) – log final (treated). Numbers in parentheses refer to standard deviation of eighteen measurements.

[b,c] Mean values in a column not followed by the same letter are significantly different ($p<0.05$).

Log reduction means of treatments at different NaCl levels were similar ($p>0.05$). In addition, there was no interaction ($p = 0.09$) between AA and NaCl levels with respect to log reductions on CF. This meant that factors AA and NaCl were independent or the effects of AA did not significantly depend ($p<0.05$) on the level of NaCl.

In general, the degree of reduction varied with the concentration of AA. This means that killing and injuring of the bacteria on CF increased with increasing AA concentration. Mean log reductions on CF using treatments A and C with 0.4% AA were significantly lower ($p<0.05$) than those from the other treatments. However, the similar mean log reduction on CF treated with 1.2 and 0.8% AA may have been related to the similar pH of processing solutions. Initial pH of solutions (before processing of CF) at 0.4, 0.8 and 1.2% AA was 3.3, 3.0 and 2.9, respectively.

In conclusion, treatment combinations of AA and NaCl using the Grovac process decreased initial counts on CF from 93.4 to 99.5%. The Grovac process seemed to be more effective in reducing the microbial counts on CF compared to other treatments using organic acids, phosphates, spray washing, either individual or combined (Table 1). Because 0.8% AA and 0.6% NaCl was effective in reducing APC counts by 2 log units (99%), it was chosen for the inoculation study discussed later.

Experiment 3. The Grovac process was evaluated for its ability to reduce *A. hydrophila* inoculated on CF. The survival and/or growth of *A. hydrophila* on refrigerated CF ($4^{o}C$) after processing and during storage are shown in Table 16. It was initially found that the APC count (log 5.5 CFU/g) of uninoculated CF (control) were slightly higher compared to the *A. hydrophila* count (log 5.2 CFU/g). These results showed that the endogenous microflora of CF were almost entirely composed of *A. hydrophila* contaminants. *A. hydrophila* are primarily aquatic organisms, they are naturally present and proliferate on aquacultured catfish. Leung *et al.* (1992b) and Fernandez *et al.* (1998b) found similar results, that catfish fillets are contaminated with *A. hydrophila* from environmental water and cross contaminated by the catfish viscera during slaughter.

The inoculated CF had an increase in APC and *A. hydrophila* counts by about 0.4 and 0.3 log CFU/g on day 0, respectively, compared to the controls. These results suggested that most of inoculated *A. hydrophila* suspension added during inoculation of

CF were killed by the Grovac process. Kirov (1995) reported that *Aeromonas* sp. are fairly sensitive to low pH (<5.5). The low pH (2.9) of the processing solution during the Grovac process could have affected the *Aeromonas* survival.

Table 16. Aerobic plate counts (APC) and *A. hydrophila* counts in inoculated and uninoculated catfish fillets (CF) treated with the Grovac process during shelf-life studies at 4°C

Treatment	Medium	Day of storage 0	3	5	7
Uninoculated	APC [a]	5.53 [d] (0.42)	6.97 [c] (0.27)	8.23 [b] (0.34)	8.51 [b] (0.15)
	A. hydrophila[a]	5.23 [e] (0.12)	6.20 [d] (0.22)	7.17 [c] (0.39)	8.03 [b] (0.32)
Inoculated	APC [a]	5.92 [e] (0.37)	6.92 [d] (0.40)	8.03 [c] (0.14)	8.90 [b] (0.27)
	A. hydrophila[a]	5.48 [d] (0.44)	6.10 [cd] (0.45)	6.75 [c] (0.20)	8.15 [b] (0.48)

[a] Mean log values measured in CFU/g. Numbers in parentheses refer to standard deviation of six measurements.

[b,c,d,e] Mean values in a row not followed by the same letter are significantly different ($p<0.05$).

Paired dependent t-test between APC means of inoculated and uninoculated CF during storage showed that they were not significantly different ($p>0.05$), except on day 7. The APC count on inoculated CF during the 7-day storage period increased significantly ($p<0.05$) by 3 log CFU/g, while the *A. hydrophila* count increased significantly ($p<0.05$) by 2.7 log CFU/g. A similar rate of microbial growth was observed on uninoculated CF during storage. The APC count on uninoculated CF during the 7-day storage period increased significantly ($p<0.05$) by 3 log CFU/g, while the *A. hydrophila* count increased significantly ($p<0.05$) by 2.8 log CFU/g. It seemed that the microbial growth during the 7-day refrigerated storage was due to the

endogenous microflora of CF composed mainly of *A. hydrophila*. Similarly, paired t-test between *A. hydrophila* means of inoculated and uninoculated CF during storage were not significantly different ($p>0.05$), except on day 5. It was also observed that pathogenic counts on inoculated and uninoculated CF did not outgrow the total counts during the refrigerated storage. This fact is benefitial from a safety standpoint because CF would undergo spoilage prior pathogen proliferation.

Both the inoculated and uninoculated CF were considered unacceptable for consumption after 3 days of storage due to the higher APC counts ($>10^7$CFU/g). However, neither inoculated nor uninoculated CF emitted a bad odor after the 7th day of storage. The inferior initial CF microbiological quality was determinant in the relatively short shelf-life of inoculated and uninoculated CF. Since psychrotrophic *A. hydrophila* are present in CF and capable of growth at refrigeration temperature, they can threaten the shelf-life and safety of chilled CF. According to Kirov (1995), *Aeromonas* sp. have been implicated in a food-poisoning outbreak after consumption of a buffet containing shrimps, smoked sausage, liver pate, and boiled ham, all of which contained a high number of *Aeromonas* (10^6 to 10^7/g of food sample). In addition, the presence of this pathogen in CF in high numbers could cause cross-contamination with cooked ready-to-eat-processed foods during market handling or in the home refrigerator.

CONCLUSIONS

A total of fifty-one bacterial isolates were isolated and identified from WC and CF using selective and nonselective culture media, phenotypic tests, and the Vitek^{TM} System (bioMérieux). From these bacteria, pathogenic microorganisms that are known to cause foodborne illness included: *Aeromonas* sp., *C. freundii, E. cloacae, E. coli, H. alvei, K. pneumoniae, Listeria* sp., *P. shigelloides, Proteus* sp., *S. aureus,* and *Vibrio* sp. Since some of these foodborne pathogens (*A. hydrophila, E. coli, Listeria* sp., *P. shigelloides, Proteus* sp., *S. aureus, V. parahaemolyticus*) are capable of growth at refrigeration temperatures, they can threaten the safety of chilled CF. In this study, endogenous *A. hydrophila* grew on CF during a 7-day refrigerated storage at 4°C.

Other bacteria isolated from WC and CF which do not represent a health hazard but they are associated with the spoilage of catfish products included: *Acinetobacter* sp., *Aeromonas* sp., *C. indologenes, C. freundii, H. alvei, Proteus* sp., *S. putrefaciens,* and *Vibrio* sp.

High APC (8.4×10^6 CFU/g) and *E. coli* (7.4×10^3 CFU/g) counts for WC indicated that catfish are highly contaminated from several potential sources, including aquatic environment, holding tanks, and processing. Similarly, APC (2.6×10^7 CFU/g) and *E. coli* (3.2×10^3 CFU/g) counts for CF indicated that fillets are contaminated during processing of catfish (e.g, deheading, skinning, eviscerating). The coliform counts and the presence of *E. coli* may indicate contamination from fecal water, or from the evisceration process. The high number of microorganisms in WC and CF along with the presence of psychrotrophic bacteria equate to the possibility of a high rate of spoilage, and thus a shorter shelf-life of chilled catfish products. CF treated with the Grovac process were considered unacceptable for consumption after 3 days of storage at 4°C due to the higher APC counts ($>10^7$CFU/g). The inferior initial CF microbiological quality and the temperature of storage were determinants in the relatively short shelf-life of CF.

The Grovac process was highly effective ($p<0.05$) reducing microbial counts on CF. The effectiveness of the process may be related to the additive effect of vacuum, tumbling, AA, and NaCl. This means that the microbial quality of CF was improved by the tumbling effect as well as the lethal and sublethal effect of the processing solution and vacuum on the endogenous microflora of CF. Treatment combinations of AA (0.4, 0.8 and 1.2%) and NaCl (0.2, 0.4 and 0.6%) decreased microbial counts on CF from 93.4 to 99.5% (log reduction from 1.2 to 2.3 CFU/g) under specific experimental conditions. Killing and injuring of the bacteria on CF increased with increased AA concentration.

The Grovac process was also challenged with CF inoculated with a *A. hydrophila* suspension (10^6 CFU/g for 15 min). It likely that most of *A. hydrophila* added by the *A. hydrophila* suspension on CF were killed by the Grovac process.

Microbiological data from this study allowed to evaluate the Grovac process on the reduction of microbial counts associated with CF at refrigerated temperatures. These data are also valuable for the hazard analysis needed during HACCP implementation of processed CF. In addition, the efficiency of the Grovac process suggests that it can be used as an alternative processing procedure to reduce microbial populations on CF and be useful to improve the shelf-life and food safety of the product. However, further research to assess the changes in physicochemical and sensory quality of CF treated with the Grovac process and stored at refrigeration temperatures are needed.

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APPENDIX: STATISTICAL ANALYSES

Paired dependent t-test of microbiological data for experiment 1

Treatment	Variable	Mean	Probability>|T|
A	Log reduction	1.22 (0.20)	0.0001
B	Log reduction	1.23 (0.23)	0.0001
C	Log reduction	1.28 (0.17)	0.0268
D	Log reduction	1.97 (0.37)	0.0001

Analysis of variance of microbiological data for experiment 2

Variable	Probability>F
Log reduction	0.0220
Ascorbic acid (AA)	0.0170
Sodium chloride (NaCl)	0.2216
AA x NaCl	0.0979

Analysis of variance of microbiological data for experiment 3

Treatment	Variable	Probability>F
Control	APC	0.0001
	A. hydrophila	0.0001
Inoculated	APC	0.0001
	A. hydrophila	0.0001

Paired dependent t-test of microbiological data for experiment 3

Treatment	Variable	Day	Mean	Probability> |T|
Control	APC	0	5.53 (0.42)	0.1245
		3	6.96 (0.26)	0.8037
		5	8.23 (0.34)	0.2308
		7	8.51 (0.15)	0.0160
	A. hydrophila	0	5.23 (0.12)	0.2305
		3	6.20 (0.22)	0.6374
		5	7.16 (0.39)	0.0497
		7	8.03 (0.32)	0.6351
Inoculated	APC	0	5.91 (0.36)	0.1239
		3	6.91 (0.39)	0.8029
		5	8.03 (0.13)	0.2156
		7	8.90 (0.27)	0.0119
	A. hydrophila	0	5.48 (0.44)	0.2094
		3	6.10 (0.45)	0.6334
		5	6.75 (0.20)	0.0411
		7	8.15 (0.48)	0.6335

VITA

Milton R. Ramos was born in Píllaro, Ecuador, on November 15, 1954. In 1984, he graduated from Universidad Técnica de Ambato, earning the degree of Ingeniero en Alimentos. In 1985, he began working as a researcher in El Instituto de Investigaciones Tecnológicas e Industriales, Universidad Técnica de Ambato. During his work, he was involved in applied research in developing of new food products. He received his master of science degree in food science from Tuskegee University, Alabama, in May 1992. In 1996, he was granted for a doctoral program in Food Science by La Fundación para la Ciencia y Tecnología de Ecuador and La Universidad Técnica de Ambato. He started his doctoral program in 1996 at Louisiana State University. His research interests focused on detection and control of pathogenic microorganisms in perishable foods. He held a graduate assistanship in the Department of Food Science, Louisiana State University, in September 1999. He is currently a candidate for the degree of Doctor of Philosophy, which will be conferred in December, 1999.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Milton R. Ramos

Major Field: Food Science

Title of Dissertation: Reduction of Endogenous Bacteria Associated with Catfish (Ictalurus punctatus) Fillets Using the Grovac Process

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

10-14-99